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## **ATTACHMENT 1: Evaluations by the External Peer Review Members**

**External Peer Reviewer: Walter J. Decker, Ph. D. F.A.A.C.T**

### **DER #1**

As a whole, this DER is acceptable. It is of concern, however, that many of the studies their in lack statistical power. This may be the reason why there is a general lack of statistical analyses in the report. The appearance of rarely-found malignant tumors in the nasal turbinates of 2 female rats should be a pointer that more animals should be tested to determine the incidence of said tumors in all dosage groups. These tumors should be further histologically defined.

### **DER #2**

It is not clear why malaxon was studied in this series of assessments of malathion. Granted malaoxon is the “active form” of malathion in the body, but it has been known for years that the biotransformation of malathion to malaxon varies in pharmacokinetics among various species.

Unless there surfaces a rational for testing malaxon in this hazard assessment of malathion, I believe DER#2 is in appropriate in this context.

### **DER#3**

The finding that the increased number of hepatocellular tumors observed in the male mice at 100 ppm as compared to the lower numbers of such tumors observed at 800 ppm is not interpretable, in my opinion. Rather, this part of the study should be repeated. The rest of the study seems to follow the Guidelines well, and appears to be scientifically valid.

### **DER#4**

I agree with the EXECUTIVE SUMMARY that this study is not acceptable and does not satisfy Guideline 83-1 for a chronic toxicity study in dogs because NOELs were not established for cholinesterase activity inhibition for plasma and erythrocytes in either sex. Therefore, it should not be used in construction a hazard assessment of malathion.

### **DER#5**

In general, this study seems to be acceptable and satisfies Guideline 83-4 for a multigenerational reproduction study in rats. There were, however, several study deficiencies pointed out by the reviewers on p.20 of the DER. The significance of these could become a debate of great magnitude, but whether they could adversely affect the study is not clear. An example is that histopathologic examinations were not performed on gross lesions of animals found dead; results of such examination on autolyzed tissue could be intermediate or downright misleading.

### **DER#6**

This DER appears to be acceptable and satisfies Guideline 83-3 for a developmental toxicity study in rats.

**DER#7**

This DER seems to be acceptable and satisfies Guideline 83-3 for a developmental toxicity study in rabbits.

**DER#8**

This DER appears to be acceptable and satisfies Guideline 83-3 for an acute delayed neurotoxicity study in hens.

**DER#9**

On p.2 of the MEMORANDUM, the phrase bilateral retinal rosette is used. This reviewer could not find rosette as a pathological term in any text consulted. What were the structures observed when submitted to histological examination? Lacking an answer to the question, I would recommend that this DER be changed from CORE MINIMUM to UNACCEPTABLE for the section on the eye histopathology. I would agree that this DER be graded core minimum for the reasons cited on p.1.

**DER#10**

Although this study appears to satisfy the requirement of Guidelines 82-7 for subchronic toxicity determinations, it was correctly pointed out in the Study Classification section that other published data indicate possible evidence of neurotoxicity on parameters not assessed in the 82-7 Guidelines. I recommend a thorough literature search on these and that the results be used to construct additional specific neurotoxicity testing to assess for effects on learning, behavior, and EEG and EMG evaluations.

**DER#11**

On p.2, the term “ratio of ratios” should be “ratios for ratios” At about the time that the Desi paper was published, William Stavinoha (University of Texas Health Science Center, San Antonio) published a paper (not available at the present time to this reviewer, who has recently moved) showing that brain cholinesterase was degraded quite rapidly in brain extraction techniques such as Desi used. This degradation was prevented by microwaving the animal’s head before the brain was removed for analyses. Using Stavinoha’s technique might provide better answers on ChE inhibition in the brain by malathion. I agree with the Footnote on page 13 that the neurotoxicity and neurobehavioral testing should be greatly expanded in scope, in light of development in these areas during the past decade. The DER should be put “on hold” until these changes are made.

**DER#12**

I agree that this DER is acceptable and satisfies Guideline 82-2 for 21 day dermal toxicity study in rabbits.

**DER#13**

It would have been less confounding if the rats were exposed to the malathion aerosols using the nose-only technique, because if rats are exposed in a whole-body chamber, they tend to lick their fur upon which the test substance adheres, thus resulting in both inhalation and oral routes of administration. Otherwise, the study design appears to be adequate. This study seems to be generally acceptable, but does not satisfy all requirements of Guideline 82-4, since, no NOEL was established for plasma and RBC cholinesterase inhibition in female animals or for microscopic lesions of the nasal cavity of the larynx in both sexes.

**DER#14**

This DER appears to be acceptable and satisfies the FIFRA Test Guideline 82-2 requirements for bacterial mutagenic data.

**DER#15**

This DER seems acceptable and satisfies the FIFRA Test Guideline 82-2b requirements for chromosome aberration data.

**DER#16**

This DER appears to be acceptable and satisfy FIFRA Test Guideline 84-4 requirements for DNA damage/repair (other genotoxicity) data.

**DER# 18**

The data in this study are considered acceptable, but do not satisfy any guideline requirement. How useful this study will be to the USEPA will largely depend on the resolution of differences of opinion between Dr. Dementi and other members of the Hazard Identification Assessment Review Committee as to whether this human study or more recent animal studies should be used to formulate USEPA policy on malathion toxicity. I recommend that Dr. Dementi's suggestions be actively pursued, that is more studies are needed to fill in the data gaps.

**DER#19**

This range -finding study is as acceptable as such. It appears that it was designated only as a range finding study, and not to be a definitive treatise on developmental toxicity of malathion to rabbits.

**DOCUMENT# 1**

This paper seems to emanate from training program for Dr. Feldman under a well known dermatotoxicologist, Dr. Maibach. It will not be useful to the USEPA in preparing a Hazard Assessment document.

**DOCUMENT# 2**

Studies #1, #2, #3, #4, and #5 are unacceptable and do not satisfy Guideline 84-2 for carcinogenicity studies in mice or rats because of deficiencies in each study. On the other hand, study #6 was well designed, and seems acceptable and satisfies Guideline 830-2 for a carcinogenicity study in ice. However, this bioassay was performed with malaxon, the active metabolite of malathion, which may dilute its impact on the Hazard Identification Assessment of malathion.

The MEMORANDUM, SUBJECT: Peer review of Malathion, dated April 12, 1990 is quite dated because of more recent research on carcinogenicity of malathion. Yet, the studies reviewed therein could certainly be used in the Hazard Assessment report.

**Selection of Principal Study**

I do not believe that the 2-year chronic feeding study in the rat should be the current selection as the Principal Study because:

1. The route of administration studies is oral, but human would be exposed primarily through inhalation or percutaneously as a result of spraying malathion.

2. Many of the studies there in lack statistical power (this may be why there is a general lack of statistical analyses in the report).

3. The appearance of rarely-encountered malignant tumors in the nasal turbinates demand further testing in a larger group of animals in all dosage groups.

Except as noted in the DER reviews, the data base seems close to complete, although the extent of the literature search is not clear. Recommend that in future reviews, reviewers be provided with a printout of "hits" in the search (at least author, title, journal, year, and abstract if available). In general the critical effects in each DER were appropriately chosen and used in the evaluation (ref.A) except as noted. I am not aware of supporting studies which shore up the use of principal study for the RfD.

## Areas I-VIII

### I Hazard Identification/Acute Oral (One-Day)

Question 1): No, for reasons cited in the reviews of DER #5 and #9.

Question 2): No, the statistical significance of maternal body weight and body weight gain is not available.

Question 3): a) A low level of confidence should be accorded this study, because:

b) The route of administration (intraperitoneal) cannot be directly compared to dietary administration. Humans are exposed to malathion generally through spraying, where inhalation and percutaneous absorption are important.

c) No, Since younger animals (and presumably younger humans are usually more sensitive, to toxic effects of any chemical, the 10X safety factor should not be discounted.

### II Determination of Susceptibility, Reproductive Toxicity

Question 1): No, because some toxic effects have been reported.

Question 2): No, because more behavioral (and learning) testing should be performed, since during the last decade, considerably more information has emerged, information which should be used to upgrade the FIFRA Guidelines.

Question 3): No because the evidence seems quite thin.

### III Hazard Identification/Chronic Dietary (RfD)

Question 1): No, because a NOEL was not identifies for erythrocyte cholinesterase inhibition among females during the first 3 months of testing.

Question 2): Flawed cholinesterase methodology is possible, but if this were true, all OP pesticides, and not just malathion, would give erroneous results.

Question 3): Additional testing should be required in the male and female rat before any thought is given to replacing the human data relied on to establish a RfD.

Question 4): I believe the 10X safety factor would be reasonable if enhanced rat data (see Question 3) were used in deriving the RfD.

Question 5): The RfD based on human data should be retained for the present time, because are apparently more sensitive.

Question 6): No, because infants and children are very likely to need additional protection, since, they are, in general, more subject to toxic insult than adults.

#### IV Subchronic Inhalation Study

Question 1): I do not feel comfortable answering this question, since the derivation of a UF of 3 is not clear to me.

Question 2): Since range-finding studies seldom generate reliable NOELs, this study should not be in deciding whether a UF should be evoked.

Question 3): Yes-common sense dictates that NOELs be identified.

Question 4): Yes, because malathion is usually sprayed, the inhalation route is most important.

Question 5): Not in my opinion! Infants and children are usually more strongly affected by toxic insults than are adults.

#### V Acute Neurotoxicity Study (Retinal Rosettes)

Question 1: Yes, histopathology data is generally desirable for all dosage groups.

Question 2: Yes, the value of independent histopathologic examination has long been recognized in clinical medicine-why not for research, also?

#### VI Subchronic Neurotoxicity Study

Question 1): An even more important reason is that during the past decade, many more neurotoxicity detection techniques have emerged and shown to be valuable. These tests were not available when the FIFRA Guideline was promulgated.

Question 2): I suggest that a neurotoxicologist be contacted to advise what criteria from published work might be used.

#### VII Cholinesterase Inhibition-Enhanced Sensitivity of Females

Question 1): There seems to be enough in the malathion data base to support the conclusion: see my

answer to Question 3.

Question 2): I can not answer this question, since it is not in my field of expertise.

Question 3) Yes, more data are needed to more rigorously define the gender-specific disparity.

### VIII Cholinesterase Inhibition-Chronic Dog Study

Question): Yes, because a NOEL is required to comply with guidelines.

### **External Peer Reviewer: Dr. Michale Dourson**

The following report places the OPP question in italicized text and the answer in bold text.

#### *Charge to the Reviewers*

Do you think the appropriate uncertainty factor was used in the establishment of the RfD?

You are asked to determine if the 2-year chronic feeding study in rat should be the correct selection as the principal study.

You are asked if the data base would be considered to be complete, and the critical effects in each DER were appropriately chosen and used in the evaluation. Are there supporting studies which shore up the use of the principal study for the RfD?

The RfD should be based on the blood cholinesterase endpoint in the 2 year bioassay of Huntingdon Life Sciences (1996) (DER # 1), where the low dietary dose of 50 ppm should be considered as a NOAEL in Fischer 344 males and female rats. The judgment that the low dose is a NOAEL, particularly in females, is based on the following 3 lines of evidence:

- i. All measurements of RBC cholinesterase inhibition are statistically unaffected at 50 ppm in both males and females at all times. Nor are the observed RBC cholinesterase inhibition measurements at 50 ppm in either males or females consistently below control values at any time, as might be expected from a real effect, but one that was not otherwise statistically significant (see table of mean cholinesterase values on page 69 of exhibit 7).
  - a)
- ii. The statistically significant effect on RBC cholinesterase inhibition in 100 ppm females at 3 months could be due to
  - chance;
  - an unusually high control value (the female control value of 1.46 IU/ml is >50% higher than the female value at 6 months and even higher than 50% when compared to the two male control values at 3 and 6 months);
  - or---the most likely explanation---the fact that the administered dose was 100 ppm in the diet for the first 3 months; since these animals would be expected to eat more diet on the basis of body weight when they are younger (i.e., =3 months), then when they are older (i.e., 3 to 6 months), their dose at =3 months at 100 ppm would also be expected to be more than the difference between 100 ppm and 50 at 6 months (e.g., 100 ppm x 0.1 food feeding factor ~ 10 mg/kg-day; while 50 ppm x 0.05 food feeding factor ~ 3 mg/kg-day).

1. The 13 week neurotoxicity rat study (DER # 10) tested a similar dose range as the 2 year bioassay and yielded similar results. Specifically, the low dose of 50 ppm diet in the 13 week study was a NOAEL in both male and female Sprague-Dawley rats.



The lack of the monitoring of the critical effect in the developing offspring, and specifically, the lack of such measurement of RBC cholinesterase inhibition in the 2 generation study is a data gap that can best be addressed through the use of a 3-fold uncertainty factor when determining the RfD. The use of this factor is well within EPA's judgment in developing RfDs, when an otherwise complete data base suggested an otherwise unmonitored endpoint that may end up being the critical effect (the effect on which the RfD is based).

I consider the FQPA safety factor as a risk management tool that should be used in the determination of risk management decisions when appropriate. Although this focus of the FQPA safety factor is helpful in assuring that sensitive subgroups—such as children on occasion—will not be missed, it is not needed in a scientific discussion of data base uncertainties and use or non-use of the data base uncertainty factor. Furthermore, the use of an additional uncertainty factor of 3-fold for the data gap specified above, precludes the use of an FQPA safety factor based on scientific reasons.

### *1 Hazard Identification/Acute Oral (One-Day)*

*Supporting documentation: DER #s 5, 6, 7, 9 and 19; References: A (pp. 3-5), B (pp. 1-4), C, D, E, V and BB (pp. 12-14; 20-22)*

*Question 1): Do the rabbit developmental toxicity and developmental range-finding toxicity studies support a conclusion that a single oral dose of malathion as high as 50 mg/kg would be without toxicological consequence in either the maternal or the developing organisms?*

The specific question to be addressed with these data are whether or not the NOEL of the likely critical effect after 1 day exposure is determinable. The available data in this review, including the developmental studies in rabbits, do not allow this question to be answered. My expectation is that the critical effect from a single day exposure is cholinesterase inhibition. I suspect that the NOEL for this endpoint after 1 day will be higher than the NOEL of 4 mg/kg-day found in the 2 year rat bioassay. The NOEL might even be as high as 50 mg/kg-day established by the i.p. study mentioned in question 3 below. The developmental studies do not measure cholinesterase inhibition. Do we not have any acute oral studies that test for it?

*Question 2): Does data on maternal body weight and body weight gain now available in Appendix III of the rabbit developmental toxicity study alter the assignment of the LOEL/NOEL for the study, and does it influence the interpretation as to whether a single dose of malathion of 50 mg/kg would be without toxicological effect?*

**The data on maternal body weight gain is potentially not relevant to the discussion of critical effect after acute exposure. Do we not have any acute oral studies that test for cholinesterase inhibition?**

*Question 3): As presented in a published work in the open literature, single intraperitoneal doses as low as 50 mg/kg in the rat reportedly elicited a clear effect on avoidance performance while cholinesterase inhibition (erythrocyte) was observed at 100 mg/kg. Plasma and brain cholinesterases were also inhibited at 150 mg/kg. Cholinesterase inhibition and decrements in behavior were all very significant though*

*transient effects: a) What level of confidence should be accorded this study?; b) What is the implication of the route of administration to the question of whether a single oral dose of 50 mg/kg can serve as the endpoint for acute dietary (one-day) risk assessment?; c) Is the data available in the developmental toxicity studies sufficiently reliable to discount the 10X safety factor required under FQPA?*

This study has the advantage of testing for what may likely be the relevant effect. Unfortunately, the route of exposure is an issue. Perhaps I missed this in the package of information for review, but do any of the available kinetic data suggest a likely speed and percent of absorption after oral exposure? If such data were available, then a crude comparison to the i.p. study might be possible, and the results of this latter study might be more relevant to the question that is posed.

I am not satisfied that the potential risk to humans is addressed with the data available in this review package. But more data are probably available to further address this question. A discussion of uncertainty factors for potential data base gaps should be postponed pending the review of these additional data.

## *II Determination of Susceptibility, Reproductive Toxicity*

*Supporting documentation: DER: # 5; References: A (pp. 15-16), B (pp. 3-4), F, G and BB (pp. 12-14; 16-17; 20-22)*

*Question 1) Can the evidence indicating greater sensitivity of offspring versus parental animals in the two-generation reproduction study in the Sprague-Dawley rat be dismissed as “...not a true indication of increased sensitivity of offspring...” for the reasons stated in the Hazard ID Committee report?*

The answer here depends in part on how the doses were calculated in the Hazard ID committee report. If the doses for the offspring were simply a reflection of the adult doses (which is what they appear to be), then the apparent greater sensitivity of offspring is offset by their likely higher ingested dose. This apparent sensitivity can thus be discounted based on the reasoning of the committee report. If instead, the offspring dose is calculated from their own food consumption, then the apparent toxicity is real and should not be discounted.

*Question 2) In the absence of assessments of cholinesterase inhibition and behavioral effects testing in adult and young animals in reproduction studies, can the data obtained in the FIFRA guideline study be considered adequate to address the question of whether young or mature animals are the more sensitive to malathion?*

No, the data on which to make this determination are absent. Perhaps the argument could be made based on analogy to other cholinesterase inhibitors that the young animals in a 2 generation reproductive study are not as sensitive, or are equally sensitive, as adults from a subchronic or 2 year bioassay. But this argument is not made in the materials provided for review.

*Question 3) Does this two-generation reproduction study provide the reliable evidence of no increased*

*sensitivity in pups when compared to adults, as required under FQPA, to discount the 10X safety factor imposed by FQPA as additional protection for infants and children?*

The absence of determining the critical effect in a potentially sensitive subpopulation necessitates the use of an additional uncertainty factor for data base deficiencies. The value of this factor should be 3-fold, in keeping with previous EPA decisions for the magnitude of such data base deficiencies. A comparison of cholinesterase inhibition between young animals and adults for other similar chemicals may provide sufficient information in order to discount the use of this additional factor.

As mentioned elsewhere in this review, I do not believe that the FQPA safety factor of 10X is appropriate in a discussion of scientific uncertainties used to establish an RfD, although the focus of the FQPA certainly helps scientists assure that sensitive subgroups, including on occasion children, are adequately addressed. As a risk management tool, the FQPA 10X may still be useful for this chemical. However, the use of an additional 3-fold factor for data base deficiencies as noted above precludes the use of an FQPA factor for scientific reasons.

### III Hazard Identification/Chronic Dietary (RfD)

*Supporting documentation: DERs: #s 1 and 10; References: A (pp. 5-6), B (pp. 4-5), H, I, N (p. 16), R and Y.*

*Question 1) Given the evidence of a post 3 months recovery of erythrocyte cholinesterase inhibition in females in the combined chronic toxicity/carcinogenicity study in the rat, can 50 ppm be concluded to have been a NOEL for the first three months of testing?*

Yes, the 50 ppm level in the chronic bioassay is a NOEL. The statistically significant effect on RBC cholinesterase inhibition in 100 ppm females at 3 months could be due to

- chance;
- an unusually high control value (the female control value of 1.46 IU/ml is >50% higher than the female value at 6 months and even higher than 50% when compared to the two male control values at 3 and 6 months);
- the fact that the administered dose was 100 ppm in the diet for the first 3 months (the most likely explanation); since these animals would be expected to eat more diet on the basis of body weight when they are younger (i.e., at 3 months), then when they are older (i.e., 6 months), their dose after 3 months at 100 ppm would also be expected to be more than the difference between 100 ppm and 50 at 6 months (e.g., 100 ppm x 0.1 food feeding factor = 10 mg/kg-day; while 50 ppm x 0.05 food feeding factor = 2.5 mg/kg-day).

*Question 2) Alternatively, do these findings suggest flawed cholinesterase methodology, and if so, what corrective measure could be pursued?*

I have no comment of the cholinesterase methodology. However, the study seems well designed and executed. The change in dose from 100 ppm to 50 ppm based on the interim results at 3 months in females seems reasonable.

*Question 3) Should 4 mg/kg/day, the NOEL for plasma cholinesterase inhibition in male, be supported as a replacement for human data previously relied upon in establishing the RfD, or should additional testing be required in the rat to identify a NOEL for cholinesterase inhibition, particularly in females?*

The additional testing is not needed. The 50 ppm level is a NOEL in females in this study. 100ppm in females is not a NOEL.

If some scientists are still motivated to pursue whether 50 ppm is or is not a NOEL in females, then a benchmark dose analysis could be done on the existing female responses, using both the 50 and 100 ppm levels (along with higher levels of course), or the results of this 2 year study could be compared with those from the 13 week neurotoxicity rat study (DER # 10) where a similar dose range as the 2 year bioassay was tested and similar results were found. Specifically, the low dose of 50 ppm diet in the 13 week study was a NOAEL in both male and female Sprague-Dawley rats.

*Question 4) Given that an explanation exists for a greater sensitivity of humans than rats with respect to cholinesterase inhibition from malathion exposure (i.e. the lack of carboxylesterase in human plasma) should a 10X safety factor applied to the rat data to allow for “uncertainties” in interspecies variability be considered adequate if the rat data is to be used in deriving the RfD?*

A crude comparison of NOEL/LOEL between the human and rat studies is possible. The male human values are 0.23 and 0.34 mg/kg-day; the rat values are 4 and 29 mg/kg-day. Ideally, one would want to match the extent and incidence of cholinesterase inhibition before making judgments about this comparison; indeed a benchmark dose applied to both data sets may allow such comparison. However, the NOELs are only ~20-fold apart; the LOELs are further, but this likely reflects the fact that the rat NOEL and LOEL was further apart than necessary in the rat study. Furthermore, the human data reflect a gavage dosing whereas the rats are continuously fed. Gavage dosing may likely lower the NOEL and LOEL when compared to continuous feeding. Thus, my overall judgment is that a 10-fold uncertainty factor applied to the rat NOEL of 50 ppm is both necessary---and satisfactory. No additional factor is scientifically needed.

*Question 5) Further, given that the RfD based on human data (0.023 mg/kg/day) is lower than that derived from the rat data (0.040 mg/kg/day) and that an explanation exists for a greater sensitivity for humans, should the RfD based on human data be retained?*

The rat study appears to be a stronger basis of the RfD than the human work. However, I believe that the rat NOEL should be further divided by a 3-fold uncertainty factor to account for deficiencies in the data base, principally because the critical effect was not monitored in the 2 generation reproductive study in a potentially sensitive subgroup (i.e., young rats). The resulting rat-based RfD is lower than the existing value on EPA's IRIS by about 50%. This comparison of rat to human RfD is thus supportive.

One might, however, ask whether a 3-fold data base factor should also be used with the human work. The use of the human data has the obvious advantage of relevance. However, it does not test females, so the NOEL/LOEL range could potentially be lower. The use of the data base factor of 3-fold would also lower this RfD. Alternatively, the human study is by gavage; thus, the NOEL/LOEL range could potentially be higher. These uncertainties might be quantifiable based on an analysis of other data for this chemical, or perhaps related chemicals, but they do operate to cancel each other out. The end result would be that the expected human-based RfD would be somewhat lower than at present, which is in the direction of the rat RfD based on an uncertainty factor of 30 (i.e., 10 for sensitive humans and 3 for data base).

*Question 6) Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10X safety factor imposed under FQPA for the protection of infants and children?*

I do not believe that the FQPA safety factor should be considered in a discussion of science behind whether a data base uncertainty factor should or should not be used. Recently I made public comments on the “use of 10x safety factor to address special sensitivity of infants and children to pesticides. I restate some of these comments here as a partial answer to question 6.

One of the intents of the FQPA is to reduce the risk to children from the noncancer effects of pesticides. This is a noteworthy goal. However, Reference Doses (or RfDs) used by EPA and others as a measure of noncancer risk are thought by many scientist to be doses below the population threshold for adverse effect<sup>1</sup>---and therefore without risk. Obviously, dividing a dose with zero risk by a safety factor of 10-fold still yields a dose with zero risk. The risk is not lessened by the use of a 10-fold safety factor.

I agree with the OPP that the FQPA safety factor of 10-fold is not part of hazard identification, nor dose response assessment. I agree that it is properly called a safety factor, and should not be considered an uncertainty factor, as established by EPA and others.<sup>1</sup> However, the use of the FQPA safety factor of 10-fold does not belong in risk characterization either. Several of the arguments listed by OPP for its use of this safety factor in risk characterization are similar---or identical---for EPA’s use of its data base uncertainty factor, used to determine RfDs.<sup>2</sup> Again the FQPA is not an uncertainty factor. It is properly seen as a safety factor and should be used by risk managers, if needed, during the risk management phase of their work.

All of EPA’s RfDs for pesticides, and many of those used by other EPA programs, protect sensitive populations---including

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<sup>1</sup> Dourson, M.L., S.P. Felter, and D. Robinson. 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. *Regulatory Toxicol. Pharmacol.* 24, 108-120.

<sup>2</sup> Dourson, M.L., Knauf, L.A. and J.C. Swartout, 1992. On Reference Dose (RfD) and Its Underlying Toxicity Data Base. *Toxicology and Industrial Health*, 8(3): 171-189.

children.<sup>3</sup> The current scheme of uncertainty factors used to develop these RfDs does not need to be expanded, although it could certainly be further studied and improved.<sup>4</sup> However, many of the older RfDs on EPA's Integrated Risk Information System (or IRIS) do not consider the data base uncertainty factor in developing RfDs. (The use of this factor was standard practice in OPP---but not the rest of the agency until about 1987.) The public health risk to children---highlighted by FQPA---should be carefully considered in reexamining these older RfDs.

#### *IV Subchronic Inhalation Study*

*Supporting documentation: DERs: #s 1, 2 and 13; References: A (pp. 9-11), B (pp. 5-6), J, N (p. 12) and O*

*Question 1) Is the use of a UF (uncertainty factor) of 3 to compensate for the absence of a NOEL for cholinesterase inhibition and nasal and laryngeal degeneration/hyperplasia supportable?*

Cholinesterase inhibition does not appear to be statistically significant at the low dose. Thus, it appears that the low dose is a NOEL for this endpoint in both males and females. ((see Table 4 of DER #13). However, the effects in the nasal passage appear to be real and biologically significant (see description and table of incidences on page 14 of DER #13). The extent and severity of these nasal lesions suggest the use of a 10-fold uncertainty factor, rather than a 3-fold factor.

OPP may wish to compare these effects after conducting dosimetry adjustments as per EPA's guidelines for the development of RfCs. This may or may not change the choice of critical effect.

*Question 2) A two-week range-finding inhalation study, evidently not available to the Hazard ID Committee, did not identify NOELs for cholinesterase inhibition or histopathology findings of nasal and laryngeal tissues at doses as low as 0.54 mg/L. Should this study influence the Hazard ID Committee decision not to evoke an uncertainty factor for acute risk assessment (i.e. 1-7 days) on the basis of cumulative effects?*

A quick comparison of LOELs for plasma or RBC cholinesterase inhibition between the two week and subchronic rat bioassays gives roughly comparable results at doses of 0.45 mg/L (subchronic) and 0.54 mg/L (2 week). The histopathology is also roughly comparable for these two doses between these two studies. Both comparisons should be checked more carefully.

I believe this supports the contention that an extra uncertainty factor for potential cumulative effects is not needed.

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<sup>3</sup> EPA. 1998. Integrated Risk Information System (IRIS). Online at [www.epa.gov/iris](http://www.epa.gov/iris).

<sup>4</sup> Swartout, J.C., Keenan, R.E., Stickney, J.A., Gillis, C.A., Dourson, M.L., Harvey, T., and P.S. Price. (In press with Risk Analysis). A probabilistic framework for the reference dose.

*Question 3) Should another study be required to identify the NOEL for the end points in question?*

I suggest that the incidence of nasal and larynx effects of similar severity (e.g., slight, moderate) be first plotted as a function of exposure (preferably after dosimetric adjustment by way of EPA's RfC methods). This may allow the use of a benchmark concentration approach to the revised incidence data. (The data shown in table 6 of DER #13 would not be useful unaltered as a basis of a benchmark concentration).

A benchmark concentration could also be applied to the cholinesterase effects in this study as well, but as stated in my answer to question 1, I believe that a NOEL for this endpoint is already demonstrated in the study.

*Question 4) Given the findings of nasal and laryngeal degeneration/hyperplasia in both of the recently submitted malathion and malaoxon combined chronic toxicity/carcinogenicity studies and the finding of rare nasal tumors in the malathion study, should the Agency require a carcinogenicity study by the inhalation route (e.g., inhalation exposure for first 90 days of a two year study)?*

Perhaps one should first ask for mechanistic studies. It may be more useful to understand the mechanism of nasal injury in eventual extrapolation (by way of a linear or a MOE approach as per draft agency cancer guidelines), rather than spend the time and money on another two year bioassay.

*Question 5) Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10X safety factor imposed under FQPA for the protection of infants and children?*

This study does not test the toxicity of malathion in rat pups or young rats, and by analogy in infants and children. See my comment also under question 6 of the previous grouping. The FQPA safety factor is a risk management tool. Its use in a scientific discussion of data base sufficiency is not appropriate. EPA has a data base uncertainty factor for such discussions.

V Acute Neurotoxicity Study (Retinal Rosettes)

*Supporting documentation: DER #s 9 and 10; References: L, M and P (pp. 1-2)*

*Question 1) Should retinal histopathology data be submitted for rats in the intermediate dose group?*

It seems reasonable to first ask for the histopathology (as in question 2 below) before requiring additional groups to be analyzed. Retinal histopathology might be a real effect, but only at high doses. Thus, effort to explore the background of this effect might be of academic interest, but have little relevance to the determination of critical effect.

*Question 2) Should histopathology slides be submitted for independent examination by the Agency's pathologist (for anatomic features comparison between control and treatment group lesion) as called for in the Data Evaluation Record (DER) for this study (a relatively simple request)?*

Yes

VI Subchronic Neurotoxicity Study

*Supporting documentation: DER #s 10, 11; References: D, P (pp. 3-4), Q, S, T, U and BB (pp. 12-14; 16-17; 20-22)*

*Question 1) Given the contrast between the NOEL of 1575 mg/kg/day (HDT) for female rats on neurotoxicity end points in this FIFRA Guideline study and that of the LOEL of 38 mg/kg/day (LDT) in the published work on a different set of neurotoxicity parameters, does the published work provide adequate reason or evidence to require a developmental neurotoxicity Guideline study or another*



*neurotoxicity study that embraces learning/memory, EEG, EMG, and possibly other neurotoxicity parameters not covered in the subchronic neurotoxicity Guideline study?*

I do not believe that it does. The LOEL of 38 mg/kg-day for both cholinesterase depression and possible learning effects is not inconsistent with the cholinesterase NOEL of 4 mg/kg-day from the 2 year rat bioassay. A division of this LOEL by a 10-fold uncertainty factor (EPA standard practice with LOELs of this degree of severity) would yield similar values (i.e., 3.8 versus 4 mg/kg-day). The use of a 3-fold uncertainty factor with this LOEL of 38 mg/kg-day would make the cholinesterase inhibition even more significant.

*Question 2) If the neurotoxicity findings in the published study are considered inadequate to trigger the additional Guideline testing, what criteria from published work, short of those upon which regulations could be directly based, might serve in that capacity? (Note: Moeller and Rider (1962), a journal publication with attendant Guideline deficiencies, has served for decades as the basis for a regulatable end point (RfD) for malathion, while the publication in question here is only being put forth as sufficiently definitive to require a study in the FIFRA Guidelines heretofore not performed.)*

Ultimately, EPA must make the judgment that the neurotoxicity findings in the published study are considered adequate to trigger the additional Guideline study. In this regard, the comments of neurotoxicologists within EPA are valuable. From my general toxicology point of view, one must also judge whether or not the expected NOEL for learning effects will be lower than the NOEL of 4 mg/kg-day for cholinesterase inhibition divided by an additional uncertainty factor of 3-fold for data base deficiencies as I suggest elsewhere in these comments. Therefore, the learning NOEL should be expected to be below ~ 1 mg/kg-day before requiring such a test. My intuition is that a learning effects NOEL would not be lower than 1 mg/kg-day, and thus an additional guideline study is not needed. Again, however, this judgment is best done by experts within EPA.

#### VII Cholinesterase Inhibition - Enhanced Sensitivity of Females

*Supporting documentation: DER #s 1 - 3, 9, 10, 12 and 13; References: W, X, Y, Z and CC*

*Question 1) Does the malathion data base support a conclusion that females are the more sensitive gender with respect to cholinesterase inhibition by this organophosphate?*

The data suggest that this might be the case in the area of dose where cholinesterase inhibition occurs. What is perhaps more important, however, is the value of the NOEL for cholinesterase inhibition in both sexes. As I have stated elsewhere in these comments, I believe that the NOEL for cholinesterase inhibition is the same for both sexes in the 2 year rat bioassay. Thus, no additional uncertainty factor is needed for this possible increased sensitivity because the RfD is based on the NOEL.

*Question 2) What approach might be taken to estimate, from the data currently available, a correction factor to be applied to the NOEL derived from the Moeller and Rider study in male human subjects to afford equivalent protection for women?*

Based on the possible difference in the extent of cholinesterase inhibition between male and female rats at the LOELs in the 2 year bioassay, the NOEL/LOEL range in female humans could potentially be lower. Alternatively, the male human study is by gavage; thus, the dietary NOEL/LOEL range in human males could potentially be higher. These uncertainties might be quantifiable based on an analysis of other data for this chemical, or perhaps related chemicals, but they do operate to cancel each other out. The end result would be that the expected human female dietary NOEL might be of the same relative value as the existing human male gavage NOEL.

I do not support the use of an additional factor to account for the potential differences in cholinesterase inhibition between sexes, because in rats, the NOELs for cholinesterase inhibition in the 2 year bioassay are the same.

*Question 3) Should additional testing in animal models be required to further quantitate the gender specific disparity?*

No. The NOELs for cholinesterase inhibition in both male and female rats are the same in the critical study.

#### VIII Cholinesterase Inhibition - Chronic Dog Study

*Supporting documentation: DER 0s 1 and 4; References: B (p. 4), H, I and AA*

*Question: Knowing that the chronic dog study has no NOEL for cholinesterase inhibition and was considered unacceptable, should additional work, e.g. subchronic feeding study, be required to characterize cholinesterase inhibition in the dog?*

We have a well characterized NOEL for cholinesterase inhibition of 4 mg/kg-day in rats of both sexes for 2 years, and a handle on where humans (the species of interest) are responding with cholinesterase inhibition from the Moeller and Rider study. With the use of an additional uncertainty factor of 3-fold for data base deficiencies applied to the rat NOEL, the RfDs from the human and rat information are quite comparable.

I do not see the need for additional testing in the dog.

**External Peer Reviewer: Rolf Hartung, Ph.D., DABT**

## PEER REVIEW OF THE RfD AND EVALUATIONS BY THE U.S. EPA'S HEALTH EFFECTS DIVISION FOR MALATHION

Reviewed by:

Rolf Hartung, Ph.D., DABT  
Professor Emeritus of Environmental Toxicology  
University of Michigan  
3125 Fernwood Ave.  
Ann Arbor, MI 48108-1955

May 29, 1998

***CHARGE TO REVIEWERS FOR THE RFD, AND EVALUATION OF MALATHION***

***And Additional Questions***

*The reviewer is asked to review each of the DERs, with the exception of the DERs pertaining to mutagenicity, and determine if the end-points were appropriately chosen and if the studies were adequately run. Was the appropriate Uncertainty Factor used in the establishment of the RfD?*

Answer:

The brief summaries of the experimental conditions that were provided are generally inadequate to determine whether the studies were appropriately conducted.

If a study was conducted using an established protocol, under careful and conscientious conditions, then the data generated by such a study are by my definition acceptable and probably useful. Judgments that are based primarily on *ex post facto* assessments of the data (e.g. was the MTD reached), should have no influence on the validity of the data, but only on their utility for a very specific purpose) (see Doc. #2).

The assessment of data cannot depend upon single pair-wise comparisons or upon differences of means or single values between experimental treatment groups and control groups. Real toxicological responses are inherently part of dose-response continua. Thus, effects noted at low levels of exposure should have counterparts of greater severity at higher dose levels. Furthermore, one would not expect to observe minute changes in responses associated with order of magnitude changes in dose levels. The only exception to the principle of stand-alone effects could occur for observations noted only at the highest dose level tested.

The assessment needs to incorporate the entire harmonized data set from all studies. It should not depend upon a search for single values, which are then treated without context.

In the case of DER #1, the selection of the plasma cholinesterase inhibition in male rats is not without problems. First of all, I would have liked to see the cholinesterase data displayed in tabular form, instead of in many paragraphs of potentially confusing text. Secondly, the dose levels between the observed LOEL and the NOEL are an order of magnitude (or more, based upon actual food intake) apart, making the estimate of the actual NOEL problematical. Mechanistically, the interest in plasma and erythrocyte cholinesterase inhibition relates to their association with the potential for inhibition of acetylcholinesterase in brain. Plasma cholinesterase has the fastest turn-over among the cholinesterases that are being discussed. Furthermore, plasma cholinesterase is synthesized in the liver, and can also be depressed as a consequence of liver damage. I find the discussion regarding the selection of plasma cholinesterase inhibition for the derivation of the RfD to be simplistic and superficial.

The level of plasma cholinesterase inhibition after Malathion (DER #1) needs to be reconciled with the observed level of plasma cholinesterase inhibition after exposure to the active compound, Malaaxon (DER #2).

*The reviewer is asked to determine if the 2-year chronic feeding study in rats should be the correct selection as the Principal Study.*

Answer:

The proposed RfD is based upon changes in plasma cholinesterase levels at 24 months, but not at earlier time points, in an extensive study in male F344 rats (DER #1), the effect was seen at much higher dose levels in female rats. The observed slope of the dose/response curve was very shallow. Human plasma cholinesterase activities regenerate fully within about 14 days, and red cell cholinesterase levels regenerate within about 100 days. Therefore the selection of the 24 month time point for the rat study does not make much sense.

Although this is an extensive study, it has been conducted in the wrong species. I clearly prefer using the existing human study, using the animal data as back-up.

*The reviewer is asked, if the data base would be considered to be complete, and if the critical effects in each DER were appropriately chosen and used in the evaluation (ref. A), are there supporting studies which shore up the use of the principal study for the RfD?*

Answer:

It would be desirable to have at least a brief discussion of the interrelations of the various cholinesterases at different sites, their functions, and their diagnostic utility in relation to OP poisoning.

As for completeness of the data base, in my opinion toxicological studies are inherently open-ended. Regardless of the existing mass of toxicological data, there are always additional questions that can be asked and the data base be further expanded, practically *ad infinitum*. Thus, there is **NO** compound that has a complete toxicological data base (I am including substances such as sugar and table salt here).

## **DETAILED REQUESTS FOR EVALUATION**

### ***I Hazard Identification/Acute Oral (One-Day)***

*Supporting documentation: DER #s 5, 6, 7, 9 and 19; References: A (pp. 3-5), B (pp.1-4), C, D, E, V and BB (pp. 12-14; 20-22)*

*Question 1): Do the rabbit developmental toxicity and developmental range-finding toxicity studies support a conclusion that a single oral dose of malathion as high as 50 mg/kg would be without toxicologic consequence in either the maternal or the developing organisms?*

Answer: DER #s 7 & 19 are repeated oral dose studies in rabbits. DER #19 is a range finding study of limited value which shows no significant effects at 50 mg/kg/day during gestation days 6-18. No significant effects were noted. DER #7 is a more complete study, also using oral doses during gestation days 6-18. This study shows increased resorption sites at 25 mg/kg. The available information is inconclusive whether a single dose, administered during a day of maximum sensitivity would be able to elicit the observed response, or whether cumulative dosing is required.

*Question 2): Do data on maternal body weight and body weight gain now available in Appendix III of the rabbit developmental toxicity study alter the assignment of the LOEL/NOEL for the study, and do they influence the interpretation as to whether a single dose of malathion of 50 mg/kg would be without toxicologic effect?*

Answer: The body weight and weight gain data can be interpreted as evidence of slight toxicological effects in the pregnant does at 50 mg/kg and higher. This evidence of maternal toxicity may be the basis for increased resorption sites at these dose levels.

*Question 3): As presented in a published work in the open literature, single intraperitoneal doses as low as 50 mg/kg in the rat reportedly elicited a clear effect on avoidance performance while cholinesterase inhibition (erythrocyte) was observed at 100 mg/kg. Plasma and brain cholinesterases were also inhibited at 150 mg/kg. Cholinesterase inhibition and decrements in behavior were all very significant though transient effects: a) What level of confidence should be accorded this study?; b) What is the implication of the route of administration to the question of whether a single oral dose of 50 mg/kg can serve as the endpoint for acute dietary (one-day) risk assessment?; c) Are the data available in the developmental toxicity studies sufficiently reliable to discount the 10X safety factor required under FQPA?*

Answer: Changes in some behavioral parameters that have a degree of correspondence to acetylcholinesterase, in particular to brain cholinesterase, would be expected. There is no requirement that the dose/response curves for acetylcholinesterase inhibition and for behavioral changes should coincide. A report by Kurtz (1977) indicates that neonate rats exhibit decrements in avoidance behavior more sensitively than adults. The biggest problem with these studies is the use of intraperitoneal dosing as a surrogate for environmentally realistic and relevant routes of exposure, which cannot be defended quantitatively. Both the distribution patterns and the pharmacokinetics associated

with intraperitoneal dosing are sufficiently different from dosing by ingestion, inhalation, or percutaneous routes, to render most quantitative assessments to be very doubtful, and to create reservations even for qualitative assessments. In most cases the i.p. route leads to more rapid absorption, and therefore higher peak blood levels than environmentally relevant exposures.

The study by Pope and Chakraborti (1992) reports higher sensitivity for cholinesterase inhibition in neonates as compared to adults. However, the study was conducted with parathion, methyl parathion and chlorpyrifos, not malathion. In the case of the mammalian toxicity of the more complex OPs (such as the phosphorodithioates), the toxicity is the result of the balance between activating and detoxifying pathways. Both types of pathways may not be fully developed in the neonate, so that it is not useful to hypothesize on the balance among these pathways for malathion in neonates based upon inferences derived from other OPs.

The additional 10X safety factor under FQPA originates in part from the inescapable higher metabolic rates and surface areas of children, which result in increased doses on a body weight basis under equivalent environmental conditions when compared to adults. The available information does not support the hypothesis that neonates are less sensitive than adults on a body weight basis. Therefore, the available information does not support deletion of the 10X FQPA safety factor.

## *II Determination of Susceptibility. Reproductive Toxicity*

*Supporting documentation: DER: # 5; References: A (pp. 15-16), B (pp. 3-4), F. G and BB (pp. 12-14; 16-17; 20-22)*

*Question 1 ) Can the evidence indicating greater sensitivity of offspring versus parental animals in the two-generation reproduction study in the Sprague-Dawley rat be dismissed as "...not a true indication of increased sensitivity of offspring..." for the reasons stated in the Hazard ID Committee report?*

Answer:

I concur with the reasoning of the Committee. But I do not concur with the ultimate conclusions with respect to safety (uncertainty) factors to be used. Whether neonates are more sensitive to a given dose of pesticide (mg/kg basis) is an important, but not the only issue. The newborn are known to have higher food intakes (on a body weight basis) than adults. The higher intake is the consequence of higher metabolic rates, due to increased heat loss determined by surface area/body weight of the neonate, and due to increased food intake due to growth requirements. For foods with equal pesticide residues the child will acquire a higher dose of pesticides than the adult (see NRC, 1993; Pesticides in the Diets of Infants and Children). Given this circumstance, it is necessary to demonstrate that the neonate is less sensitive (not equal to) than the adult on a mg/kg basis, or that food pesticide residues are being managed in such a way as to restrict the quantities of residues likely to be found in the diet of children as compared to adults.

*Question 2) In the absence of assessments of cholinesterase inhibition and behavioral effects testing in adult and young animals in reproduction studies, can the data obtained in the FIFRA guideline study be considered adequate to address the question of whether young or mature*

*animals are the more sensitive to malathion?*

Answer:

The comparison of relative sensitivities is only meaningful when data exist that were collected under comparable conditions. It is obviously possible to make many comparisons, as long as comparably derived data exist. However, because toxicity testing is inherently open ended, a question of this type can never be answered with certainty in detail, although general comparisons are possible.

*Question 3) Does this two-generation reproduction study provide the reliable evidence of no increased sensitivity in pups when compared to adults, as required under FQPA, to discount the 10X safety factor imposed by FQPA as additional protection for infants and children?*

Answer:

That is not the correct question, the appropriate question should be whether this study provides clear evidence of less sensitivity among pups as compared to adults - for reasons cited elsewhere in this report.

### *III Hazard Identification/Chronic Dietary (RfD)*

*Supporting documentation: DERs: #s 1 and 10; References: A (pp. 5-6), B (pp. 4-5), H, I, N (p. 16), R and Y.*

*Question 1) Given the evidence of a post 3 months recovery of erythrocyte cholinesterase inhibition in females in the combined chronic toxicity/carcinogenicity study in the rat, can 50 ppm be concluded to have been a NOEL for the first three months of testing?*

Answer:

No. Once the malathoxon-acetylcholinesterase bond is established in RBCs, the rate of recovery is identical to the red cell replacement rate, which is approximately 1%/day.

*Question 2) Alternatively, do these findings suggest flawed cholinesterase methodology, and if so, what corrective measure could be pursued?*

Answer:

This requires an analysis of the detailed cholinesterase methodology. The cholinesterases as well as many other esterases are surprisingly non-specific. Which substrates were used? What was the pH and temperature? Were substrate and product inhibition avoided? (See G. B. Koelle, 1963. Cholinesterases and anticholinesterase agents. Handbuch der experimentellen Pharmakologie, Ergänzungswerk, Vol. 15. Springer Verlag, Berlin)

*Question 3) Should 4 mg/kg/day, the NOEL for plasma cholinesterase inhibition in males, be supported as a replacement for human data previously relied upon in establishing the RfD, or should additional testing be required in the rat to identify a NOEL for cholinesterase inhibition, particularly in females?*

Answer:

No. The human is the correct species of concern. Substituting a rodent introduces many more

uncertainties than those produced by minor deficits in the analysis of chemical purity or concerns about statistical precision.

*Question 4) Given that an explanation exists for a greater sensitivity of humans than rats with respect to cholinesterase inhibition from malathion exposure (i.e. the lack of carboxylesterase in human plasma) should a 10X safety factor applied to the rat data to allow for "uncertainties" in interspecies variability be considered adequate if the rat data is to be used in deriving the RfD?*

Answer:

Look at what you are doing! Here you are willing to accept a study for which you are also willing to mess around with another factor of 10X, just because the statistical data are neater. In the process you are willing to discount human data, even though it is extremely unlikely that the equivalent statistical uncertainties for the human will reach anywhere close to 10X !

*Question 5) Further, given that the RfD based on human data (0.023 mg/kg/day) is lower than that derived from the rat data (0.040 mg/kg/day) and that an explanation exists for a greater sensitivity for humans, should the RfD based on human data be retained?*

Answer:

YES

*Question 6) Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10X safety factor imposed under FQPA for the protection of infants and children?*

Answer:

See previous discussions related to the 10X FQPA safety (uncertainty?) factor.

#### IV Subchronic Inhalation Study

*Supporting documentation: DERs: #s 1, 2 and 13; References: A (pp. 9-11), B (pp. 5-6), J. N (p 12) and O*

*Question 1 ) Is the use of a UF (uncertainty factor) of 3 to compensate for the absence of a NOEL for cholinesterase inhibition and nasal and laryngeal degeneration/hyperplasia supportable?*

Answer:

This fine-tuning is unwarranted because of major species differences in exposure scenarios. In whole body exposures of rodents we deal with a direct nasal inhalation, an inhalation filtered through fur as rats assume a characteristic protective fetal position during the exposure, and a direct ingestion as rats seek to clean their fur by licking it. This is not comparable to the exposure scenario of the human applicator, the bystander, or the consumer.



The unique nasal responses of rats have been discussed previously during the assessment of the inhalation toxicity of formaldehyde.

*Question 2) A two-week range-finding inhalation study, evidently not available to the Hazard ID Committee, did not identify NOELs for cholinesterase inhibition or histopathological findings of nasal and laryngeal tissues at doses as low as 0.54 mg/L. Should this study influence the Hazard ID Committee decision not to invoke an uncertainty factor for acute risk assessment (i.e. 1-7 days) on the basis of cumulative effects?*

Answer:

The same objections raised for Question IV-1 still apply here.

*Question 3) Should another study be required to identify the NOEL for the end points in question?*

Answer:

Not with rats on these issues.

*Question 4) Given the findings of nasal and laryngeal degeneration/hyperplasia in both of the recently submitted malathion and malaoxon combined chronic toxicity/carcinogenicity studies and the finding of rare nasal tumors in the malathion study, should the Agency require a carcinogenicity study by the inhalation route (e.g., inhalation exposure for first 90 days of a two year study)?*

Answer:

There are so many questions regarding the validity of whole body exposures that their validity for human health risk assessments will remain questionable. Nose-only studies impose so many additional stresses on the rat, that the interpretation of any results also becomes seriously confounded.

*Question 5) Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10X safety factor imposed under FQPA for the protection of infants and children?*

Answer:

No

#### V Acute Neurotoxicity Study (Retinal Rosettes)

*Supporting documentation: DER #s 9 and 10; References: L, M and P (pp. 1-2)*

*Question 1) Should retinal histopathology data be submitted for rats in the intermediate dose group?*

Answer:

Yes, for qualitative analysis, only. The reason for this is that either the dosing schedule or the cholinesterase analyses were deficient in this study. But, retinal detachments as potential end-points are sufficiently serious to merit following up on the present anecdotal finding.

*Question 2) Should histopathology slides be submitted for independent examination by the Agency's pathologist (for anatomic features comparison between control and treatment group lesion) as called for in the Data Evaluation Record (DER) for this study (a relatively simple request)?*

Answer:

They should be examined. Whether by the mechanism in question 1 or 2 does not particularly concern me at this time.

#### VI Subchronic Neurotoxicity Study

*Supporting documentation: DER #s 10, 11; References: D, P (pp. 3-4), Q, S, T, U and BB (pp. 12-14; 16-17; 20-22)*

*Question 1) Given the contrast between the NOEL of 1575 mg/kg/day (HDT) for female rats on neurotoxicity end points in this FIFRA Guideline study and that of the LOEL of 38 mg/kg/day (LDT) in the published work on a different set of neurotoxicity parameters, does the published work provide adequate reason or evidence to require a developmental neurotoxicity Guideline study or another neurotoxicity study that embraces learning/memory, EEG, EMG, and possibly other neurotoxicity parameters not covered in the subchronic neurotoxicity Guideline study?*

Answer:

Neurotoxicity studies are generally fairly easy to conduct, but they tend to present major difficulties in the selection of proper controls and in the interpretation of the results, especially between different species. The studies in DER #10 and DER #11 show no behavioral effects at dose levels significantly above dose levels associated with plasma cholinesterase inhibition, but they do show abnormalities in EEG and EMG recordings after 90 days of exposure.

Historically, Russian scientists have relied heavily upon neurophysiological measurements in the assessment of toxicity. These studies have generally not been accepted in western nations, mainly because of difficulties with interpreting the data. If this issue is to be reconsidered, then it would require an in-depth assessment of the the under-lying science, and its utility for developing acceptable exposures. Furthermore, such an approach would need to be extended to most pesticides.

The spread between simple behavioral responses and cholinesterase inhibition argues against a need for further study. But, the sensitivity of behavioral studies can vary significantly, depending upon the end-point that is measured.

I am not familiar with the full range of studies that could be asked for under a 'developmental neurotoxicity Guideline' study. If this is an unrestricted list of test methods, then it should be subjected to an assessment of interpretability and utility prior to requiring its execution.

*Question 2) If the neurotoxicity findings in the published study are considered inadequate to trigger the additional Guideline testing, what criteria from published work, short of those upon which regulations could be directly based, might serve in that capacity? (Note: Moeller and Rider (1962), a journal publication with attendant Guideline deficiencies, has served for decades as the basis for a regulateable end point (RfI) for malathion, while the publication in question*

*here is only being put forth as sufficiently definitive to require a study in the FIFRA Guidelines heretofore not performed.)*

Answer:

There is a major difference between the Moeller and Rider study and DER #10 & 11. The Moeller and Rider study is conducted in the proper species, and it addresses a diagnostic end-point that is known to be mechanistically related to the toxicity of OPs. DER #11 (Dési et al.) is based upon EEG frequency band ratios and upon the EMG frequency of unit potentials. I see no difference in the difficulties of interpretation of this study and a voluminous body of Russian neurotoxicological literature that we have rejected historically.

### VII Cholinesterase Inhibition - Enhanced Sensitivity of Females

*Supporting documentation: DER #s 1 - 3, 9, 10, 12 and 13; References: W, X, Y, Z and CC*

*Question 1 ) Does the malathion data base support a conclusion that females are the more sensitive gender with respect to cholinesterase inhibition by this organophosphate?*

Answer:

The data were not assembled in a way that would allow ready assessment of this question. The emphasis in the data presentation for the present review centers on NOELs and LOELs, etc., not on the actual dose response data (corrected for actual consumptions), which should be plotted and tested for differences. The available information should be found in the supporting data for DER #1 for rats, DER #3 for mice, DER #4 for dogs, DER #9 for rats, DER #10 for rats, DER #12 for rats (percutaneous absorption, use with caution). These data, especially as summarized in Refs. W, Y, and Z, indicate that females compared to males are in some cases approximately equally sensitive to cholinesterase inhibition, and in other cases are more sensitive. I was unable to find any rigorous statistical comparisons of the underlying dose/response relationships.

DER #2 does not contribute to this question, it was derived for malaoxon.

*Question 2) What approach might be taken to estimate, from the data currently available, a correction factor to be applied to the NOEL derived from the Moeller and Rider study in male human subjects to afford equivalent protection for women?*

Answer:

I am not aware of any direct data that show that the human female is more sensitive to cholinesterase inhibition for equivalent doses than males are. Therefore, the simplest first step is to explore whether the existing data indicate whether gender-mediated sensitivities are universal across the species that have been tested to date. If so, then what is the magnitude (ratio) of the difference? If a ratio can be discerned and defended, then it can be applied to the data applicable for the human male.

*Question 3) Should additional testing in animal models be required to further quantify the gender specific disparity?*

Answer:

1.- Do a thorough analysis of existing data first, as suggested for question 2.

- 2.- Add another species, if necessary.
- 3.- If possible, expand the Moeller study to include females.

#### VIII Cholinesterase Inhibition - Chronic Dog Study

*Supporting documentation: DER #s 1 and 4; References: B (p. 4), H. I and AA*

*Question: Knowing that the chronic dog study has no NOEL for cholinesterase inhibition and was considered unacceptable, should additional work, e.g. subchronic feeding study, be required to characterize cholinesterase inhibition in the dog?*

*Answer:*

No, the study is perfectly adequate for the detailed analysis of the dose/response curves for cholinesterase inhibition. As a matter of fact, the elusive NOEL is practically useless for a rigorous dose/response analysis to explore gender-mediated or species-mediated differences in sensitivity.

## **ATTACHMENT 2: HIAR REPORT OF 12/17/97**

HED Doc. No. 012440

**Date:**       **December 17, 1997**

### **MEMORANDUM**

**SUBJECT:**   **MALATHION:** - Report of the Hazard Identification Assessment Review Committee.

**FROM:**       Jess Rowland  
                  Branch Senior Scientist,  
                  Science Analysis Branch, Health Effects Division (7509C)

**THROUGH:** K. Clark Swentzel, Chairman,  
                  Hazard Identification Assessment Review Committee  
                  Toxicology Branch II, Health Effects Division (7509C)  
                  And  
                  Mike Metzger, Co-Chairman  
                  Hazard Identification Assessment Review Committee  
                  Reregistration Action Branch 2, Health Effects Division (7509C)

**TO:**           Al Nielsen, Branch Senior Scientist  
                  Reregistration Branch 2  
                  Health Effects Division (7509C)

**PC Code: 057701**

On November 6, 1997, the Health Effects Division's Hazard Identification Review committee evaluated the toxicology data base, selected doses and endpoints for acute dietary, chronic dietary (RfD) as well as occupational and residential exposure risk assessments, and addressed the sensitivity of infants and children from exposure to Malathion as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report.

### Committee Members in Attendance

Members in attendance were Karl Baetcke, William Burnam, George Ghali, Karen Hamernik, Susan Makris, Nancy McCarroll, Mike Metzger, Kathy Raffaele, John Redden, Jess Rowland and Clark Swentzel. Member in absentia: Melba Morrow. Data was presented by Ed Budd of Registration Action Branch 2.

HED staff members also participating at the meeting were: Brian Dementi, Toxicology Branch 1, William Sette, Science Analysis Branch and, Paula Deschamp (Exposure Scientist), Diana Locke (Risk Assessor), and Pauline Wagner (Chief, Reregistration Action Branch 2).

Data Presentation: \_\_\_\_\_  
Ed Budd, M.S

Report Preparation: \_\_\_\_\_  
Jess Rowland, M.S

## I. INTRODUCTION

On November 6, 1997, the Health Effects Division's Hazard Identification Review committee evaluated the toxicology data base to select the doses and endpoints for acute dietary, chronic dietary (RfD) as well as occupational and residential exposure risk assessments, and addressed the enhanced sensitivity of infants and children from exposure to Malathion as required by the Food Quality Protection Act (FQPA) of 1996.

## II. HAZARD IDENTIFICATION

### A. Acute Dietary (one-day)

Study Selected: Range-Finding Developmental Toxicity - Rabbit and  
Developmental Toxicity - Rabbit §83-3

MRID Nos. 00152569 (Range Finding) 40812001 (Main Study)

Executive Summary: Range Finding: In a Range-Finding study, pregnant New Zealand white rabbits (5/group) received oral administration of Malathion (92.4%) in corn oil at doses of 0, 25, 50, 100, 200, or 400 mg/kg/day on Gestation Days (GD) 6-18. No mortalities or clinical signs were observed at 25, 50 or 100 mg/kg/day. At 200 mg/kg/day, 2 does died, 1 on GD 11 (5 days after dosing) and another on GD 17 (11 days after dosing). At 400 mg/kg/day, 4 does died, 1 on GD 7, 1 on GD 8 and 2 on GD 9. Cholinergic signs of toxicity seen at 200 and 400 mg/kg/day included tremors, decreased activity and salivation. External examinations of the fetuses did not indicate any gross abnormalities. For Maternal Toxicity, the NOEL was 100 mg/kg/day and the LOEL was 200 mg/kg/day based on mortality and clinical signs.

Main Study: In a prenatal developmental toxicity study, pregnant New Zealand White rabbits (20/group) received oral administration of Malathion (92.4%) in corn oil at doses of 0, 25, 50, or 100 mg/kg/day on gestation days 6-18. Mortality in 2 does at 100 mg/kg/day were attributed to dosing (gavage) errors. For Maternal Toxicity, the NOEL was 25 mg/kg/day and the LOEL was 50 mg/kg/day based on reduced mean body weight gains during treatment. During the dosing period (Days 6-18), mean body weight gains were 0.19, 0.06, -0.03 and -0.03 kg at 0, 25, 50 and 100 mg/kg/day, respectively. Anorexia and soft stools were seen at all doses including the controls, but occurred at a slightly higher incidence at 100 mg/kg/day. For Developmental Toxicity, the NOEL was 25 mg/kg/day and the LOEL was 50 mg/kg/day based on a slightly increased incidence of mean resorption sites per dam.

Dose and Endpoint for Risk Assessment: NOEL of 50 mg/kg/day. The Committee selected this dose based on a weight-of-the-evidence consideration from the Range-Finding

and the Main Study as well as pertinent information from other studies.

Comments about Study and Endpoint: In the Range-Finding study no deaths occurred at 100 mg/kg/day. Death attributable to a single dose (i.e., the period of exposure of concern) occurred only in 1 doe on the first day of dosing (GD 7) at 400 mg/kg/day. At 200 mg/kg/day deaths occurred only after multiple doses (i.e., GD 11 and 17)]. No treatment-related mortality was seen in the main study. None of the clinical signs (anorexia and soft stool) seen in both studies were attributable to a single exposure.

In the Main Study, the decrease in mean body weight gain in does at 50 mg/kg/day (LOEL) observed during the dosing period was not attributable to a single dose but rather to multiple doses. It should be noted no mortalities, clinical signs or decreases in body weight gain were seen at the same dose (50 mg/kg/day) in the Range-Finding study. Thus, toxicological endpoints (e.g., death, clinical signs) attributable to a single dose were not observed at 50 mg/kg/day. The increase in resorption sites/dam at 50 mg/kg/day was not considered to be an appropriate endpoint because the incidence was only slightly increased and was considered by the Committee to be of no meaningful toxicological significance with respect to acute dietary risk assessment. Based on a weight-of-the-evidence consideration from the Range-Finding and the Main Study and other pertinent information from other studies on Malathion, the Committee determined that a NOEL of 50 mg/kg/day is appropriate for acute dietary risk assessment.

The Committee did not consider the acute neurotoxicity study in rats (MRID No. 43146701) to be appropriate because of low confidence in the assessment of cholinesterase activity. In rats given a single oral dose of Malathion at 0, 500, 1000 or 2000 mg/kg, plasma and erythrocyte cholinesterase were inhibited in both sexes at 2000 mg/kg on Day 7, a finding which was sustained, in females only on Day 15. Also, there was equivocal inhibition of plasma cholinesterase in females at 500 and 1000 mg/kg which was characterized by a poor dose response. No inhibition of brain cholinesterase activity was seen in either sex at any dose level. Thus the lack of dose response and a clear NOEL for this biomarker constituted an inherent weakness of this study since inhibition of cholinesterase activity was seen in other studies among various species (rats, and dogs) at much lower doses.

**This risk assessment is required.**

Acute Dietary Risk Assessment: The Committee determined that the **10 x** factor to account for enhanced sensitivity of infants and children (as required by FQPA) **should be removed**. For acute dietary risk assessment, a **Margin of Exposure (MOE) of 100 is adequate** for the protection of the general U.S. population including infants and children from acute exposure to Malathion. A MOE of 100 is adequate because.

- (I) Developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and



- rabbits.
- (ii) A two generation reproduction toxicity study in rats showed no increased sensitivity in pups when compared to adults.
  - (iii) The toxicology data base is complete and there are no data gaps.

## **B. Chronic Dietary [Reference Dose (RfD)]**

§83-5 Study Selected: Combined chronic Toxicity/carcinogenicity -Rat Guideline

MRID No. 43942901

Executive Summary: In a combined chronic toxicity/carcinogenicity study, groups of Fischer 344 rats (90/sex/dose) were fed diets containing Malathion (**97.1%**) at 0, 100/50, 500, 600 or 12000 ppm (equivalent to 0, 4, 29, 359 or 739 mg/kg/day in males and 0, 5, 35, 415 or 868 mg/kg/day in females, respectively) for up to 24 months. The low dose of 100 ppm was reduced to 50 ppm after 3 months due to inhibition of erythrocyte cholinesterase activity in females. For chronic toxicity, the NOEL was 50 ppm (4 mg/kg/day) and the LOEL was 500 ppm (29 mg/kg/day) based on inhibition of plasma cholinesterase activity in males at 24 months.

Dose and Endpoint for establishing the RfD: NOEL=4 mg/kg/day based on significant inhibition of plasma cholinesterase activity at 29 mg/kg/day (LOEL).

Uncertainty Factor (UF): An UF of 100 was applied to account for inter (10 x)-and intra- (10 x) species variation.

$$\text{RfD} = \frac{4 \text{ mg/kg/day (NOEL)}}{100} = \mathbf{0.04 \text{ mg/kg/day}}$$

Comments about Study and Endpoint: The RfD derived from the use of the NOEL and endpoint from the above animal study and an Uncertainty Factor of 100 is supported by a comparable RfD that could have been derived from the use of the NOEL from a human study and an Uncertainty Factor of 10.

In a 1962 study conducted with male human volunteers, Malathion (**purity not known**) was administered by gelatin capsule once each day to groups of 5 healthy male volunteers ranging in age from 23 to 36 years. Based on an assumed body weight of 70 kg, the dosage regimen was 0.11 mg/kg/day for 32 days, 0.23 mg/kg/day for 47 days and 0.34 mg/kg/day for 56 days. Plasma and erythrocyte cholinesterase activities were determined twice weekly before, during and after administration. Some of the volunteers were also given another test material (EPN) alone or in combination with various doses of Malathion

over the course of the study. No clinical signs or symptoms of toxicity were observed at any dose level at any time. The NOEL was 0.23 mg/kg/day and the LOEL was 0.34 mg/kg/day based on inhibition of plasma and erythrocyte cholinesterase.

When the **NOEL** of 4 mg/kg/day **from an animal** study is used in conjunction with an **Uncertainty Factor of 100** (10 x for inter-species and 10 x for intra-species variations), the **RfD** derived is **0.04 mg/kg/day**.

When the **NOEL** of 0.23 mg/kg/day **from a human study** is used in conjunction with an **Uncertainty Factor of 10** (for intra-species variation), the **RfD** derived is **0.02 mg/kg/day**.

The Committee decided to use the animal study instead of the human study for deriving the RfD for the following reasons: 1) low confidence in the human study due to the use of only one sex (males), unknown purity of Malathion, and the unavailability of raw data for proper evaluation); 2) the completeness of the animal toxicology data base, known purity (97.1%) of Malathion tested and the NOEL of the 2-year study supported by the results in a 13-week neurotoxicity study in rats in which the NOEL for inhibition of cholinesterase activity was also 4 mg/kg/day in both males and females.

Chronic Dietary Risk Assessment: The Committee determined that the **10 x** factor to account for enhanced sensitivity of infants and children (as required by FQPA) **should be removed**. For chronic dietary risk assessment, **a UF of 100 is adequate** for the protection of the general U.S. population including infants and children from chronic exposure to Malathion. A UF of 100 is adequate because.

- (I) Developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits.
- (ii) A two generation reproduction toxicity study in rats showed no increased sensitivity in pups when compared to adults.
- (iii) The toxicology data base is complete and there are no data gaps.

## **C. Occupational/Residential Exposure**

### **1. Dermal Absorption**

Study: Published Study (Feldman, RJ and Maibach, HI. (1970)

Executive Summary: In this dermal absorption study in humans, <sup>14</sup>C-radiolabeled Malathion (dissolved in acetone) was applied to a 13 sq cm circular area on the ventral surface of the forearms of 7 subjects at a rate of 4 ug/sq cm. The skin sites were not protected. All urine was collected for 5 days and assayed for radioactivity in a liquid scintillation counter. Dermal penetration of Malathion through the skin was estimated by calculating the total amount of radioactivity excreted in the urine in 5 days. A mean of 7.84%  $\pm$ 2.71% (SD) of the applied dose of radioactivity was recovered in the 5 day urine, indicating a dermal absorption rate of approximately 5% to 10% over a 5 day period. Based on the above, the Committee concluded that the dermal absorption rate is about 10%.

Dermal Absorption Factor: A dermal absorption factor of 10% should be used for converting oral dosing to dermal dosing.

Comments about Study: The dermal absorption rate of 10% established in the human study is supported by the dermal absorption (DA) calculated by comparing the NOELs or LOELs in the oral developmental toxicity study and the 21-day dermal toxicity study in the same species (rabbits) as shown below

Type of Study	NOEL	LOEL	Estimated DA based on NOELs	Estimated DA based on LOELs
Main Study-Developmental	25	50	$25 \div 1000 = 2.5\%$	$50 \div 1000 = 5\%$
Range-Finding-Developmental	100	200	$100 \div 1000 = 10\%$	$200 \div 1000 = 20\%$
21-Day Dermal Toxicity	1000	>1000		

Additional support for the dermal absorption rate of 10% in humans is provided in a study by Castles and Reddy. In that study, human percutaneous absorption was determined for Malathion (neat), Ortho Malathion 50 (50% Malathion in Xylene), Ortho Malathion diluted to 1% in water and Ortho Malathion diluted in 10% water when applied to the forearm. The mean doses applied were 0.8, 0.9, 0.032 and 1.13 mg/sq cm. respectively. Mean absorption, based on urinary excretion of label, for a 24 hour exposure was 7.2, 5.6, 15.0 and 5.5, respectively (Castles and Reddy, January, 1993; Tox.Doc.No. 011314).

## **2. Short-Term Dermal - (1-7 days)**

Study Selected: 21-Day Dermal Toxicity - Rabbit      Guideline

§82-2

MRID No 41054201

Executive Summary: Groups of New Zealand White rabbits (6/sex/dose) received 15 repeated dermal applications of Malathion (94%) at 0, 50, 300 or 1000 mg/kg/day, 6 hours/day, 5 days/week over a three week period. Except for one death at the high dose due to acute mucoid gastroenteritis, no mortality occurred. No treatment-related effects were seen in body weight, body weight gain, food consumption, clinical signs, hematology or clinical chemistry parameters, organ weights and gross or histopathology. Slight dermal irritation was seen at application sites. The overall NOEL was 50 mg/kg/day and the LOEL was 300 mg/kg/day based on significant inhibition of plasma and red blood cell cholinesterase activity in both sexes and in the brain of females.

Dose and Endpoint for Risk Assessment: NOEL = 50 mg/kg/day based on significant inhibition of plasma, red blood cell and brain cholinesterase activity at 300 mg/kg/day (LOEL).

Comments about Study and Endpoint: Inhibition of cholinesterase activity following oral administration was also observed following dermal applications, the route of exposure of concern.

**This risk assessment is required.**

### **3. Intermediate-Term Dermal (7 Days to Several Months)**

Study Selected: 21-Day Dermal Toxicity - Rabbit      Guideline  
§82-2

MRID No      41054201

Executive Summary: See Short-Term

Dose and Endpoint for Risk Assessment: NOEL=50 mg/kg/day based on significant inhibition of plasma, red blood cell and brain cholinesterase activity at 300 mg/kg/day (LOEL).

Comments about Study and Endpoint: The Committee selected this dose for this risk assessment, since the NOEL of 50 mg/kg/day established following dermal exposure in the 21-day dermal study is supported by the NOEL of 4 mg/kg/day established following oral exposure in the 13-week neurotoxicity study in rats when a dermal absorption factor of 10% is applied. In both studies (i.e. via both routes), the LOEL was based on a common toxicological endpoint, inhibition of plasma, red blood cell and brain cholinesterase activity.

In a subchronic neurotoxicity study (MRID No. 43269501), Sprague-Dawley rats received dietary administration of Malathion at 0, 4, 352 or 1486 mg/kg/day to males and at 0, 4, 395 or 1575 mg/kg/day to females for 13 weeks. For cholinesterase activity, the NOEL was 4 mg/kg/day in both sexes and the LOEL was 352 mg/kg/day in males and 395 mg/kg/day in females based on inhibition of plasma and erythrocyte cholinesterase activity in both sexes and on inhibition of brain cholinesterase activity in females.

Application of a dermal absorption rate of 10% to the oral NOEL of 4 mg/kg/day yields a comparable dermal dose of 40 mg/kg/day ( $4 \text{ mg/kg/day} \div 10\% \text{ DA} = 40 \text{ mg/kg/day}$ ). Thus the 40 mg/kg/day is analogous to the 50 mg/kg/day NOEL observed in the 21-day dermal study in rabbits based on the same toxicological endpoints.

**This risk assessment is required.**

#### **4. Long-Term Dermal (Several Months to Life-Time)**

Study Selected: Combined Chronic Toxicity/Carcinogenicity - Rat §83-5

MRID No 43942901

Executive Summary: See Chronic Dietary

Dose and Endpoint for Risk Assessment: NOEL=4 mg/kg/day based on significant inhibition of plasma cholinesterase activity at 29 mg/kg/day (LOEL).

Comments about Study and Endpoint: This dose and endpoint was used in establishing the RfD. Since an oral dose was selected, a dermal absorption rate of 10% should be used in dermal risk assessments. When a dermal absorption rate of 10% is applied to the oral NOEL of 4 mg/kg/day, a comparable dermal dose of 40 mg/kg/day is obtained (i.e.,  $4 \text{ mg/kg/day} \div 10\% \text{ DA} = 40 \text{ mg/kg/day}$ ).

**This risk assessment is required.**

#### **5. Inhalation Exposure (Any-Time period)**

Study Selected: 90-Day Inhalation -Rat Guideline: 82-4

MRID No 43266601

Executive Summary: In a subchronic inhalation study, groups of Sprague-Dawley rats (15/sex/concentration) were exposed in whole body inhalation chambers to

Malathion (96.4%) at aerosol concentrations of 0.1, 0.45, or 2.01 mg/L for 6 hours/day, 5 days/week for 13 weeks. Treatment had no effects on survival, body weights or food consumption. Cholinergic signs observed at 2.01 mg/L and sporadically in a few animals at the lower doses included red staining of the urogenital areas, excess salivation and ungroomed oily fur. Treatment-related histopathological lesions were seen in the nasal cavity and the larynx of both sexes of rats at all concentrations tested. The lesions in the nasal cavity were characterized as slight to moderate degeneration and/or hyperplasia of the olfactory epithelium which was locally extensive. The lesions of the larynx were characterized as epithelial hyperplasia, with squamous keratinization occurring in some rats. In addition, the olfactory/respiratory epithelial junction was severely affected in most animals. For systemic toxicity, a NOEL was not established and the LOEL was 0.1 mg/kg/day based on histopathologic lesions of the nasal cavity and larynx. Inhibition of plasma and red blood cell cholinesterase activity was seen in female rats at all concentrations. In male rats, inhibition of cholinesterase activity was observed in plasma at 2.01 mg/L and in red blood cells at  $\geq 0.45$  mg/L. Inhibition of brain cholinesterase activity was seen only at the highest concentration. For overall cholinesterase inhibition, a NOEL was not established for plasma and red blood cells; the LOEL was 0.1 mg/L. For inhibition of brain cholinesterase, the NOEL was 0.45 mg/L and the LOEL was 2.01 mg/L.

Dose and Endpoint for Risk Assessment: LOEL = 0.1 mg/L based on inhibition of plasma and red blood cell cholinesterase activity and histopathological lesions of the nasal cavity and larynx at the lowest concentration tested.

Comments about Study and Endpoint: Since this is the only inhalation study that is available in the toxicology data base, the LOEL from this study will be used for Short-, Intermediate-and Chronic inhalation risk assessments.

**This risk assessment is required.**

#### **D Margin of Exposure for Occupational/Residential Exposures:**

##### **1. MOE for Dermal Exposures**

For Short-, Intermediate- and Long-Term dermal exposures **a MOE of 100 is adequate** for occupational and residential exposures to Malathion via the dermal route because:

- (I) Developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits.

- (ii) A two generation reproduction toxicity study in rats showed no increased sensitivity in pups when compared to adults.
- (iii) The toxicology data base is complete and there are no data gaps.

## 2. MOE for Inhalation Exposures

The dose (0.1 mg/L) selected for inhalation risk assessments is a LOEL and thus ordinarily would necessitate the use of an additional Uncertainty Factor (UF) for risk assessments (under FIFRA). For Malathion, however, an additional UF of 3 should be applied only for Intermediate and Long-Term but not for Short-Term exposure risk assessments.

The Committee determined that an additional UF of 3 is not required for Short-Term risk assessments because the toxicological endpoints (inhibition of plasma and red blood cell cholinesterase activity and histopathological lesions) seen at the lowest concentration tested are considered to be cumulative effects (the result of multiple dosing) and are not expected to occur after 1-7 days of treatment (the Short-Term exposure period of concern). In the acute neurotoxicity study, no cholinesterase inhibition was seen after a single oral dose except at a very high dose. In subchronic and chronic studies via the oral route, inhibition of cholinesterase activity has been observed only after repeated dosing with Malathion.

Therefore, the Committee determined that **a MOE of 100 is adequate** for **Short-Term exposure** risk assessments but **a MOE of 300 is required** for **Intermediate-and Long-Term exposure** risk assessments for exposures to Malathion via inhalation. The additional UF of 3 is applied under FIFRA because a LOEL was used for these risk assessments. No FQPA factors are required since there was no indication of increased sensitivity in the offspring of rats or rabbits in prenatal exposure studies on Malathion.

## **E. CLASSIFICATION OF CARCINOGENIC POTENTIAL**

On September 24, October 8 and 15, 1997 HED's Cancer Assessment Review Committee (CARC) evaluated the carcinogenic potential of Malathion. The CPRC reviewed the following studies: 1) Carcinogenicity study in B6C3F1 mice with Malathion; 2) Combined chronic toxicity/carcinogenicity study in Fischer 344 rats with Malathion; and 3) Combined chronic toxicity/carcinogenicity study with Malaoxon in Fischer 344 rats. The CARC recommended re-evaluation of certain tissues/slides from these studies since an assessment on the relevancy of observed tumors to treatment could not be made due to the absence of critical histopathological data. The CARC re-affirmed the current classification of Malathion as a **Group D** Carcinogen - Not Classifiable as to Human Carcinogenicity (Memorandum: J. Rowland SAB to M. Ioannou, TB2, November 3, 1997).

## **F. MUTAGENICITY**

### **(I). Gene Mutation:**

In a *Salmonella typhimurium*/*Escherichia coli* reverse gene mutation assay, Malathion (95.2%) was non-mutagenic when tested at concentrations up to 5000 µg/plate (highest dose tested) with or without S9 activation (MRID No. 40939302).

### **(ii). Chromosome Aberrations:**

In an *in vivo* bone marrow cytogenetic assay, Malathion (94%) was negative following oral doses at 500-2000 mg/kg to male and female Sprague-Dawley rats. A dose-related reduction in mitotic indices (MIs) was seen in the females of all treatment levels at 24 hours. Reduced MIs were also recorded for high-dose males and females at 48 hours (MRID No. 41451201).

### **(iii). Other Mutagenic Effects:**

In an *in vitro* primary rat hepatocytes unscheduled DNA synthesis (UDS) assay, Malathion (94%) was negative up to cytotoxic levels (0.12 µL/mL; . 150 µg/mL).(MRID No. 41389301).

### **(iv). Other Information:**

Under the pre-1991 guidelines, the three acceptable studies [*S.typhimurium*/*E. coli* reverse gene mutation assay, *in vivo* bone marrow cytogenetic assay in rats, and unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes (UDS)] satisfy the minimum requirements in the three major categories of genetic testing. The acceptable studies were negative. However, an open literature review of mutagenicity studies on Malathion and Malaoxon, a metabolite formed by oxidation, was prepared for the Carcinogenicity Peer Review of Malathion held on February 7, 1990 (see Memorandum from K. Dearfield to J. Edwards, 1990).

In addition, the mutagenicity potential of Malathion was again evaluated by HED's CPSC on September 24 and October 8 and 15, 1997. The overall assessment indicated that there is overwhelming confirmation from the published literature demonstrating that Malathion is genotoxic, producing structural damage to chromosomes *in vitro* and in whole animal studies with mice and hamsters. Similar conclusions were reached by Flessel et al., (1993) in the genetic toxicology review prepared for the California Department of Health Services. No assays with germinal cells have been submitted to the Agency. However, Malathion was negative in *Drosophila melanogaster* sex-linked recessive lethal assays, mouse dominant lethal assays and spermatogonia and/or spermatocyte cytogenetic assays.



A questionable clastogenic response was reported in mouse spermatocytes following subacute exposure to commercial grade Malathion (Salvadori *et al.*, 1988). Nevertheless, the data from reproduction toxicity (MRID No. 41583401) and developmental studies (00152569, 41160901) and epidemiological surveys of pregnant women exposed to Malathion (Arevalo *et al.*, 1987; Spielman, 1986; Grether, *et al.*, 1987) do not suggest adverse heritable effects. The Committee concluded, therefore, that requiring studies in germinal cells was not warranted.

No mutagenicity studies have been submitted to the Agency on Malaoxon. The consensus opinion from the above cited reviews of the open literature is that Malaoxon is not mutagenic in bacteria but is positive without S9 activation in the mouse lymphoma assay forward gene mutation assay. Malaoxon was not clastogenic in cultured Chinese hamster ovary (CHO) cells; however, the findings from the mouse lymphoma assay suggest that Malaoxon may induce both gene mutations and chromosome aberrations. Nonactivated Malaoxon also caused SCEs in independently performed investigations with CHO cells.

(iv). Conclusions:

The positive mutagenicity studies with Malathion support the evidence of liver tumor induction in male and female mice. Based on the overall results, there is a clear concern for somatic cell mutagenicity. No further testing is required since all mutagenicity issues have been addressed.

**G. Recommendation for Aggregate Exposure Risk Assessments**

For aggregate exposure risk assessment, the MOEs derived for the oral, dermal and inhalation exposures may be combined to obtain a total MOE since a common toxicological endpoint (i.e., cholinesterase inhibition) was observed following exposure via these routes in oral, dermal and inhalation toxicity studies.

$$\frac{1}{\text{MOE}_{\text{Oral}}} + \frac{1}{\text{MOE}_{\text{Dermal}}} + \frac{1}{\text{MOE}_{\text{Inhalation}}}$$

**III. FQPA CONSIDERATIONS**

1. Neurotoxicity Data

In an acute delayed neurotoxicity study, Malathion (93.6%) was administered by gavage to

atropinized hens at 1007.5 mg/kg (1.3 x the oral LD50 of 775 mg/kg). A second dose (852.5 mg/kg) was given by gavage at study day 21. Mortality was extensive (only 14/60 hens survived the full study). Clinical signs of neurotoxicity, considered to be due to inhibition of cholinesterase activity, were observed for up to 6 days after dosing. No further clinical signs or gross or microscopic evidence of neuropathology was observed. (MRID No. 40939301)

In an acute neurotoxicity study, groups of Sprague-Dawley rats (27/sex/dose) received a single oral administration of Malathion (96.4%) in corn oil at doses of 0, 500, 1000, or 2000 mg/kg. For neurotoxicity, the NOEL was 1000 mg/kg and the LOEL was 2000 mg/kg/day based on decreased motor activity at peak effect time (day 1) and clinical signs (salivation, body staining, one death with tremor, labored breathing, stained fur, decreased defecation and urination). Plasma and erythrocyte cholinesterase were inhibited in both sexes at 2000 mg/kg on Day 7, a finding which was sustained, in females only on Day 15. Also, there was an equivocal inhibition of plasma cholinesterase for females at 500 and 1000 mg/kg, characterized by a poor dose response. No inhibition of brain cholinesterase activity was seen in either sex at any dose level. Equivocal neuropathological findings at 2000 mg/kg included axonal degeneration in the lumbar root and bilateral retinal rosette in one male, digestion chambers in the lumbar dorsal root fibers in one male and in the sciatic and tibial nerve in another male rat. The one rat with bilateral retinal rosette was observed among but five males examined histopathologically in the high dose group, and that none were examined in lower dose groups. Digestion chambers and axonal degeneration of the sciatic nerve were also seen in one male control rat. (MRID No. 43146701).

In a subchronic neurotoxicity study, groups of Sprague-Dawley rats (25/sex/dose) were fed diets containing Malathion (96.4%) at 0, 50, 5000 or 20,000 ppm (0, 4, 352, or 1486 mg/kg/day in males and 0, 4, 395, or 1575 mg/kg/day in females, respectively). For systemic toxicity, the NOEL was 5000 ppm (352/395 mg/kg/day for M/F) and the LOEL was 20,000 ppm (1486/1575 mg/kg/day in M/F) based on decreased body weight and food consumption and on increased clinical signs (anogenital staining, and dried red material around the nose). For cholinesterase inhibition, the overall NOEL was 50 ppm (4 mg/kg/day) and the LOEL was 5000 ppm (352/395 mg/kg/day in M/F) based on inhibition of plasma and red blood cell cholinesterase in males and females and on Brain cholinesterase in females. There were no treatment-related effects on brain weight or neuropathology (MRID No. 43269501))

In a published study by Desi *et al.* (1976) Malathion (95%) was administered to female CFY rats at dietary doses of 38 and 75 mg/kg/day for 90 days. The authors reported that maze performance was affected during the first 21 days of the study and EEG and EMG recordings were affected after 90 days. Brain cholinesterase activity was inhibited, but clinical signs of cholinergic poisoning were not observed during the study.

In an *ad hoc* meeting of HED neurotoxicity experts convened to consider this study (and certain other studies on Malathion), the consensus of the meeting participants was to perform a literature search on this finding and related findings for organophosphates in general and for Malathion in particular and if warranted by new information, consider requesting additional neurotoxicity studies on Malathion. The conclusions of this *ad hoc* meeting is attached to this Report.

## 2. Determination of Susceptibility

There is no indication of additional sensitivity to young rats or rabbits following pre-and/or postnatal exposure to Malathion in the developmental and reproductive toxicity studies.

### (I) Developmental Toxicity:

In a prenatal developmental toxicity study, pregnant Sprague-Dawley rats (25/group) received oral administration of Malathion (94%) in corn oil at doses of 0, 200, 400, or 800 mg/kg/day during gestation days 6 through 15. For maternal toxicity, the NOEL was 400 mg/kg/day and the LOEL was 800 mg/kg/day based on decreased body weight gain, decreased food consumption and clinical signs of toxicity (urine-stained abdominal fur). No developmental toxicity was observed. For developmental toxicity, the NOEL was  $\geq 800$  mg/kg/day (HDT); a LOEL was not attained. Neither maternal nor fetal cholinesterase levels were measured in this study. (MRID No. 41160901)

In a prenatal developmental toxicity study, pregnant New Zealand White rabbits (20/group) received oral administration of Malathion (92.4%) in corn oil at doses of 0, 25, 50, or 100 mg/kg/day during gestation days 6 through 18. For maternal toxicity, the NOEL was 25 mg/kg/day and the LOEL was 50 mg/kg/day based on reduced mean body weight gains during treatment. For developmental toxicity, the NOEL was 25 mg/kg/day and the LOEL was 50 mg/kg/day based on a slightly increased incidence of mean resorption sites per dam. Neither maternal nor fetal cholinesterase levels were measured in this study. (MRID No. 40812001).

In a Range-Finding study, pregnant New Zealand white rabbits (5/group) received oral administration of Malathion (92.4%) in corn oil at doses of 0, 25, 50, 100, 200, or 400 mg/kg/day on Gestation Days (GD) 6-18. No mortalities or clinical signs were observed at 25, 50 or 100 mg/kg/day. At 200 mg/kg/day, 2 does died, 1 on GD 11 and another on GD 17. At 400 mg/kg/day, 4 does died, 1 on GD 7, 1 on GD 8 and 2 on GD 9. Cholinergic signs of toxicity seen at 200 and 400 mg/kg/day included tremors, decreased activity and salivation. External examinations of the fetuses did not indicate any gross abnormalities. For Maternal Toxicity, the NOEL was 100 mg/kg/day and the LOEL was 200 mg/kg/day based on mortality and clinical signs (MRID No. 00152569).

(ii) Reproductive Toxicity:

In a two-generation reproduction study, groups of Sprague-Dawley rats (25/sex/group) were fed diets containing Malathion (94.0%) at concentrations of 0, 550, 1700, 5000, or 7500 ppm (43, 131, 394, or 612 mg/kg/day in males and 51, 153, 451, or 703 mg/kg/day in females, respectively). For parental systemic toxicity, the NOEL was 5000 ppm (394/451 mg/kg/day in M/F) and the LOEL was 7500 ppm (612/703 mg/kg/day in M/F) based on decreased P generation body weights during gestation and lactation and decreased F1 pre-mating body weight. No effects on reproduction were observed. For offspring toxicity, the NOEL was 1700 ppm (131/153 mg/kg/day in M/F) and the LOEL was 5000 ppm (394/451 mg/kg/day in M/F), based on decreased F1a and F2b pup body weights during lactation. In the F1b and F2a litters, the pup weight decrements were observed at 7500 ppm (612/703 mg/kg/day). Although the DER describes this as a developmental NOEL/LOEL, the only treatment-related Day 0 body weight decrease in pups occurs at 7500 ppm in the F1b litters. In fact, pup body weight decrements were primarily observed at postnatal day 21. Neither adult nor offspring cholinesterase was measured (MRID No. 41583401).

Although the offspring NOEL (131 mg/kg/day in males and 153 mg/kg/day in females) was lower than the parental systemic NOEL (394 mg/kg/day in males and 451 mg/kg/day in females), the Committee determined that this was not a true indication of increased sensitivity of offspring because: (i) pup body weight decrements were primarily observed at postnatal day 21; (ii) during that period (i.e., later portion of lactation), young rats consume approximately twice the diet per unit body weight as an adult rat consumes (i.e., 1 ppm in the diet of a young rat is approximately 0.1 mg/kg/day whereas in older rats, this ppm level is equal to 0.05 mg/kg/day) and (iii) the estimation of the test substance intake in pre-weaning animals is likely to be more than double the adult intake because of the availability of the test material both via the milk (lactation) and food, particularly after the mid point of lactation.

(iii). Information from the Open Literature:

These summaries are provided to develop a comprehensive picture of Malathion toxicity. The data have not been reviewed in depth, and no statement is made regarding the accuracy or quality of the data or reports.

In day 1-3 chicken embryos, Malathion appears to produce multiple malformations of the wing-level and trunk/leg level spinal cord, eye, tailbud, and cardiovascular system, some of which result from aberrations in the neural fold, with from 125 µg to 4 mg Malathion (Wytttenbach and Thompson, 1985).

Neurotoxic esterase and delayed neuropathology studies in hens were judged not to demonstrate a potential for Malathion to cause delayed neurotoxicity. (Erich et al, 1995 and

Jianmongkol et al, 1996)

Oral administration of Malathion at 0, 10 or 20 µg on gestation days 6, 9, or 12 to white mice did not result in developmental toxicity (Mufti and Safdar, 1991).

A study of Malathion exposure to sheep (20 mg/kg/day) on gestation months 3-5 resulted in ataxia, hind leg weakness and depression in the dams and abortion, still births, dystocia, placental retention, and low birth weight lambs. (Thatoo and Prasad, 1988).

A case study demonstrated delayed neurotoxicity in a suicide attempt exposure to Malathion (100 ml of 50% Malathion); findings may have been exacerbated by chronic alcoholism. (Komori et al, 1991).

A case study suggested a relationship between Malathion exposure (via head lice treatment shampoo during the 11th to 12th week of pregnancy) and a malformation of the nervous system development (amyoplasia-like condition) in an infant (Lindhout and Hageman, 1987).

An *in vitro* study of human fetal brain and liver suggested that "Malathion "altered the level of enzymes associated with glutathione cycle and antioxidase defense system", involving "alterations in glutathione status and extent of lipid peroxidation." The effect was greater in brain tissue than liver, and greater with earlier developmental stage, suggesting to the authors that there is a greater susceptibility of the human fetus to Malathion (Gupta et al, 1991).

Epidemiological surveys of pregnant women exposed to Malathion in Chile (Arevalo et al, 1987), Germany (Spielman, 1986), and the San Francisco area (Thomas et al, 1987; Grether, et al., 1987) suggested no adverse effects. A preliminary review of the study by Thomas and Green indicates that the San Francisco studies included groups of large sample sizes (7,450 and 22,465 births) which presumably should have resulted in statistically robust conclusions.

### 3. Recommendation for a Developmental Neurotoxicity Study

The Committee determined that, based on a weight-of-the-evidence review of the available data, a developmental neurotoxicity study with Malathion in rats is not required at this time. The following information was considered in arriving at this decision.

(I) Evidence that support requiring a developmental neurotoxicity study:

# Malathion is a neurotoxic organophosphorus pesticide. Administration to

various species (human, rat, mouse, dog) causes inhibition of cholinesterase activity in various compartments.

- # Some equivocal neuropathology was observed in the perfused tissues from the acute neurotoxicity study in rats.
- # Minimal equivocal learning and memory effects were observed in the study in rats by Desi *et al.*
- # A case study from the open literature indicated that delayed neuropathy resulted from a suicide attempt in an adult human male with chronic alcoholism. (This study was not supported, however, by other data in the literature or by the results of animal studies.)

(ii) Evidence that do not support asking for a developmental neurotoxicity study:

- # No evidence of abnormalities in the development of the fetal nervous system, were observed in the prenatal developmental toxicity studies in either rats or rabbits, at maternally toxic oral doses up to 800 or 100 mg/kg/day, respectively.
- # Neither brain weight nor histopathology (perfused or nonperfused) of the nervous system was affected in subchronic and chronic toxicity studies in several species, and in the neurotoxicity studies in rats.
- # Available epidemiological data did not find adverse effects associated with exposure of pregnant human females to Malathion.

#### 4. Determination of Uncertainty Factor:

The Committee determined that for Malathion, the **10 x factor** to account for enhanced sensitivity of infants and children (as required by FQPA) **should be removed**. This conclusion was based on the following factors.

- (I) Developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits.
- (ii) A two generation reproduction toxicity study in rats showed no increased sensitivity in pups when compared to adults.

- (iii) The toxicology data base is complete and there are no data gaps.

#### IV. DATA GAPS

The toxicology data base is complete for Malathion; there are no data gaps.

#### V. OTHER ISSUES

##### A. Resolution of Issues Related to Neurotoxicity

The Committee determined that an *ad hoc* group should resolve three outstanding issues related to the neurotoxicological testing of Malathion. The three issues identified were:

- 1). The possibly greater sensitivity of females (as compared to males) to the cholinesterase inhibiting effects of Malathion, and how this sex difference might affect the RfD for this chemical
- 2). Should the Agency require the Registrant to submit the microscopic slides (or photomicrographs) of retinal tissue from three rats in the acute and subchronic neurotoxicity studies on Malathion?
- 3) Should the Agency require the Registrant to perform and submit additional neurotoxicity studies to evaluate possible effects of Malathion on learning and/or behavior and/or other neurotoxicological parameters as exemplified in a literature article by Desi et al. (1976) in which maze performance (learning) and EEG and EMG recordings were reported as being affected in rats treated with Malathion?.

The conclusions of the *ad hoc* group meeting of November 13, 1997 are in Attachment 1.

##### B. Minority Reports

Three "Memorandums" from Brian Dementi, Toxicologist, to Clark Swentzel, Chairman, Hazard Identification Assessment Review Committee dated November 10, November 20, November 25, and one Memorandum from Brian Dementi, Toxicologist, to Jess Rowland, Executive Secretary, Hazard Identification Assessment Review Committee dated December 11, 1997 are in Attachment 2.

## **VI SUMMARY OF TOXICOLOGY ENDPOINT SELECTION**

The doses and toxicological endpoints selected as well as Margins of Exposures (MOE's) for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY	MOE
Acute Dietary	NOEL =50.0	Maternal toxicity	Range-Finding & Main Developmental toxicity studies - rabbits	100
Chronic Dietary	NOEL=4.0	Inhibition of plasma cholinesterase activity	Combined/Chronic Toxicity Carcinogenicity - Rat	UF= 100
Short-Term (Dermal)	NOEL =50.0	Inhibition of plasma, RBC and brain cholinesterase activity	21-Day Dermal - Rabbit	100
Intermediate-Term (Dermal)	NOEL=50.0	Inhibition of plasma, RBC and brain cholinesterase activity	21-Day Dermal - Rabbit	100
Long-Term (Dermal)	NOEL=4.0	Inhibition of plasma cholinesterase activity	Combined/ Chronic Toxicity - Rat	100
Short-Term (Inhalation)	LOEL= 0.1 mg/L	Inhibition of plasma, and RBCcholinesterase activity & histopathology in respiratory epithelium	90-Day Inhalation Toxicity	100
Intermediate-Term (Inhalation)	LOEL= 0.1 mg/L	Inhibition of plasma, and RBCcholinesterase activity & histopathology in respiratory epithelium	90-Day Inhalation Toxicity	300
Long-Term (Inhalation)	LOEL= 0.1 mg/L	Inhibition of plasma, and RBCcholinesterase activity & histopathology in respiratory epithelium	90-Day Inhalation Toxicity	300



## ATTACHMENT 1 FOR THE 12/17/97 HIARC REPORT

Malathion: Report on the *ad hoc* Neurotoxicity Subgroup Meeting of November 13, 1997

December 3, 1997

### MEMORANDUM

SUBJECT: Malathion: Report on the ad hoc Neurotoxicity Subgroup Meeting of November 13, 1997

DP Barcode D240967      Pesticide Chemical No. 057701  
(Subbean to D238907)      Tox Chemical No. 535  
Case 818961  
Submission S529758

FROM: Edwin R. Budd, Toxicologist  
Registration Action Branch 2  
Health Effects Division (7509C)

TO: Clark Swentzel, Chairman  
Hazard ID SARC  
Health Effects Division (7509C)

THRU: Richard Loranger, Branch Senior Scientist  
Registration Action Branch 2  
Health Effects Division (7509C)

### INTRODUCTION

At the request of the Hazard ID SARC, which met on November 6, 1997 to conduct a toxicological assessment on malathion, an ad hoc neurotoxicity subgroup was formed to consider and resolve three outstanding issues related to the neurotoxicological testing of this chemical. The seven persons comprising this subgroup were nominated by Clark Swentzel and Mike Ioannou on November 6, 1997 and were the following: Clark Swentzel, William Sette, Kathleen Raffaele, Robert Fricke, Virginia Dobozy, Brian Dementi, and Edwin Budd (all staff members of HED). The

subgroup met on November 13, 1997 from 1:00 to 3:15 PM. This report presents the decisions of the subgroup and will be appended to the final Hazard ID SARC report.

ISSUE #1--The possibly greater sensitivity of females (as compared to males) to the cholinesterase inhibiting effects of malathion, and how this sex difference might affect the RfD for this chemical.

Discussion: On November 6, 1997, the Hazard ID SARC decided to base the RfD for malathion on the results of the 2-year combined chronic feeding/carcinogenicity study on rats (MRID 43942901). For the purpose of setting the RfD, the SARC considered the NOEL for inhibition of cholinesterase activity in this study to be 50 ppm in the diet (equivalent to 4 mg/kg/day in males and 5 mg/kg/day in females). A 32-56 day oral study in humans (males only)(Moeller and Rider, 1962) with a NOEL for inhibition of cholinesterase activity of 0.23 mg/kg/day was also discussed by the Hazard ID SARC and considered to be supportive of the RfD.

Subsequent to the November 6, 1978 meeting and during the neurotoxicity subgroup meeting on November 13, 1997, the issue was raised as to whether it would have been more appropriate to base the RfD for malathion on the results of the human study, rather than on the rat study. After considerable discussion, Clark Swentzel, in the capacity of chairman of the Hazard ID SARC, agreed to discuss this matter with selected members of the SARC to determine whether or not the full SARC might or might not be asked to readdress the choice of studies on which the RfD for malathion is based.

Regarding the possibly greater sensitivity of females (as compared to males) to the cholinesterase inhibiting effects of malathion, the results of cholinesterase determinations in numerous studies on malathion were discussed and it was agreed that females do indeed appear to be more sensitive than males. There was not full agreement, however, on the relative degree of increased sensitivity of females compared to males. Also, there was not full agreement on whether or not a modifying factor should be applied to the RfD for malathion if the human study (in which only males were tested) were eventually selected to be the study on which the RfD for malathion were based.

Recommendation: The consensus of the neurotoxicity subgroup was that if the human study were eventually chosen as the basis for the RfD, it would not be appropriate to apply an additional modifying factor to the RfD to account for the increased sensitivity of females as compared to males. The rationale for this recommendation was that although a sex difference in sensitivity apparently does exist, the difference appears to be small. In many (but not all) studies, the sex difference did not result in different cholinesterase NOELs for males and females, but rather in different degrees of cholinesterase inhibition for males and females at a given dose level. It was pointed out that NOELs, rather than degrees of effect

at a given dose level, are used in HED to determine RfDs and as the basis for various other risk assessment calculations. It was also pointed out that this same issue (possibly greater sensitivity of one sex) had arisen several times in the past with respect to setting the RfD for other chemicals and that as a general policy it had previously been decided that additional modifying factors based on possible sex differences ordinarily would not be applied to RfDs.

The neurotoxicity subgroup also agreed that if the 2-year combined chronic feeding/carcinogenicity study in rats were retained by the Hazard ID SARC as the basis for the RfD, the question of whether or not to apply an additional modifying factor based on sex to the RfD would be "moot" since 50 ppm (equivalent to 4 mg/kg/day in males and 5 mg/kg/day in females) was the cholinesterase NOEL for both males and females in the study.

ISSUE #2--Should EPA require the registrant to submit the microscopic slides (or photomicrographs) of retinal tissue from three rats in the acute and subchronic neurotoxicity studies on malathion?

Discussion: In the draft DER for the acute neurotoxicity study in rats (MRID 43146701), it was observed that 1/5 high dose group male rats had a bilateral retinal "rosette". Since concerns had arisen in recent years regarding the possibility that exposure to malathion might affect the visual system of humans and/or experimental animals, and since treatment-related lesions of the visual system had been observed in studies with certain other organophosphate pesticides, the occurrence of the bilateral retinal "rosette" in this high dose animal was considered by the reviewer to be a potentially serious effect of the test material and to warrant full investigation into the pathology and possible cause of the lesion in this animal. Further, the lesion was most likely a very rare event in rats. Toward this end, several pathologists were contacted regarding the potential seriousness of this lesion. These pathologists included Dr. Lucas Brennecke (EPA consulting pathologist), Dr. Robert Dahlgren (the study pathologist) and Dr. C. B. Clifford (Charles River pathologist). In addition, in the past, considerable discussion of this matter among several HED staff members also occurred, but all without resolution of the question of whether or not to ask the registrant to provide the microscopic slides of the retina of this rat to EPA for further examination--together with the slides of the retina of a control rat in the subchronic neurotoxicity study (MRID 43268501) which showed a unilateral retinal "rosette" and the slides of the retina from a randomly selected control rat from the acute study. Since the term "rosette" lacks histopathological preciseness, the slides of the retina of the control rat were required to determine if the lesion in this animal was indeed the same or was different than that in the high dose animal. Prior to the neurotoxicity subgroup meeting, additional information on retinal rosettes derived from a National Library of Medicine literature search was provided by Virginia Dobozy. The neurotoxicity subgroup discussed all the available

information and data.

Recommendation: The consensus of the neurotoxicity subgroup was that, based on the presently available information, EPA should not ask for the microscopic slides of the retinas of these three rats at this time. The rationale for this recommendation included a weight-of-the-evidence consideration of the following:

The lesion of concern (bilateral retinal rosette) occurred in only one high dose male rat in the acute neurotoxicity study.

A unilateral retinal rosette was also tentatively observed in one control male rat in the subchronic neurotoxicity study.

Drs. Brennecke and Dahlgren both concluded the retinal rosette in the high dose male rat was not of toxicological significance and was not due to treatment with malathion.

Dr. Dahlgren considered the cause to be a "developmental deficit which occurs at the time of retinal maturation".

The neurotoxicity subgroup also concluded that retinal rosettes in rats are most likely the result of abnormal proliferation and differentiation of developing retinal cells during neonatal life (i.e. during the first approximately 32 days after birth) and ordinarily are not likely to develop in mature animals as a result of treatment with xenobiotics.

In a reference book available to the subgroup (Ophthalmic Pathology of Animals, Saunders and Rubin, 1975), it was stated that "[Retinal] rosettes occur spontaneously in certain strains of inbred rats and in beagle and collie dogs."

ISSUE #3--Should EPA require the registrant to perform and submit additional neurotoxicity studies to evaluate possible effects of malathion on learning and/or behavior and/or other neurological parameters as exemplified in a literature article by Desi et al. (1976) in which maze performance (learning) and EEG and EMG recordings were reported as being affected in rats treated with malathion?

Discussion: In the subchronic neurotoxicity study in rats (MRID 43269501), a guideline study that included a "functional observational battery" (FOB) and motor activity measurements, treatment-related effects on these two parameters were not observed at the highest dose level tested--20000 ppm (equivalent to 1486 mg/kg/day in males and 1575 mg/kg/day in females). However, in a non-guideline subchronic neurotoxicity study in

female rats (reported by Desi et al., 1976), which employed dose levels of 0, 38 and 75 mg/kg/day, malathion was reported to affect maze performance (learning/memory) during the first 21 days of the study (increased errors and increased running time) and to affect EEG and EMG recordings after 90 days. At the dose levels tested in the Desi et al. study, brain cholinesterase activity was inhibited about 20% at 21 days, but clinical signs of cholinergic poisoning were not observed. Therefore, learning/memory deficits and changes in EEG and EMG recordings were reported in the absence of cholinergic clinical signs (i.e. at subclinical doses). Since the guideline subchronic neurotoxicity study (MRI(D 43269501) did not assess either learning/memory or EEG or EMG effects, it was recommended in the draft DER that the registrant be required to perform and submit additional neurotoxicity studies on malathion to evaluate possible effects on learning/behavior and EEG and EMG changes. A schedule-controlled operant behavior study (guideline 85-5) was suggested as a possibility. The neurotoxicity subgroup discussed the general subject of learning/behavior studies and also considered specific information pertinent to the Desi et al. study. In addition, a memorandum from R.C. MacPhail (Chief, Neurobehavioral Toxicology Branch/HERL/EPA) to John Doherty (HED) and Brian Dementi (HED), dated May 4, 1995, was available which commented on the Desi et al. study and on the potential regulatory usefulness of further neurotoxicity testing of malathion as recommended in the draft DER.

Recommendation: The consensus of the neurotoxicity subgroup was that, based on the presently available information, EPA should not ask for additional neurotoxicity studies on malathion at this time. It was recognized, however, that such studies might possibly be requested at some time in the future if there were sufficient justification for doing so. Toward this end, the subgroup suggested it would be appropriate to perform a literature search on 1) learning/ behavior effects of organophosphates in general, and 2) available information on malathion in particular. After the literature search was completed and if warranted by new information, the question of additional neurotoxicity testing for malathion might be reconsidered.

cc: Brian Dementi  
William Sette  
Kathleen Raffaele  
Robert Fricke  
Virginia Dobozy  
Mike Ioannou  
Diana Locke  
Jess Rowland

ATTACHMENT -2 FOR THE 12/17/97 HIARC REPORT

Memorandum -1: From Brian Dementi to Clark Swentzel, November 10, 1997.

Memoradum: From Brian Dementi to Clark Swentzel, November 20, 1997

Memorandum: Brian Dementi to Clark Swentzel, November 25, 1997

Memorandum: From Brian Dementi to Jess Rowland, December 17, 1997



HazardID SARC  
Health Effects Division

As a follow-up to the November 6, 1997 HazardID SARC on malathion, I am compelled to express in writing my disagreement with certain very important decisions rendered at that meeting. One such issue concerns the apparent decision of the Committee to shift the basis of the RfD for malathion from the NOEL in the human study (Moeller and Rider, 1962), which has served in this capacity for years, to the NOEL for cholinesterase inhibition in the 1996 F344 rat chronic toxicity/carcinogenicity study. The problems I have with this decision are developed as follows. Firstly, the decision was too precipitous. By this I mean that since this is such a critical end point for this pesticide, it should have been presented as an issue or topic well before the meeting to allow people to be better prepared for discussion. I view this as a problem inherent in the process in dealing with a chemical having an extensive scientific record. Accordingly, there must be opportunity for offering further arguments supportable by additional information.

To the extent that Moeller and Rider incorporates a valid assessment of the LOEL/NOEL for cholinesterase inhibition in human subjects, being based as it is on **both** plasma and erythrocyte cholinesterases, evidence suggests humans are at least 10-fold more sensitive than F344 rats for erythrocyte cholinesterase inhibition and even more sensitive with respect to the plasma enzyme. To explain this difference, someone at the meeting suggested that 1962 vintage malathion was of questionable purity and that impurities could explain the differences with respect to the 1996 product. However, it was not indicated that humans have historically been more sensitive, i.e. were more sensitive than rat as compared on the basis of earlier products and likely remain so as compared to the more recent Cheminova product. Critical to the sensitivity of organisms to malathion in the cholinergic sense is the presence and level in such organisms of carboxylesterase activity, an enzyme(s) which, via catalysis of hydrolysis of one carboxyethyl group on malathion (actually malaoxon as the cholinesterase inhibiting entity), compromises its cholinesterase inhibitory capabilities. As I indicated at the meeting, insects lack carboxylesterase activity, which is thought to explain the remarkable selective efficacy of malathion as an insecticide. Similarly, to the extent that mammals incorporate differential levels of carboxylesterase activity they are variably sensitive to the agent in the cholinergic sense. Published works show that while carboxylesterase activity is located in the plasma and liver of the rat, in humans the enzyme is found in liver but not plasma. (Exhibit 1) The greater sensitivity of humans as demonstrated in Moeller and Rider may have its explanation in differing carboxylesterase activity in man versus rat. However, whatever the explanation, the fact remains that Moeller and Rider demonstrates the greater sensitivity of humans as compared historically using malathion of existing purity at the time and would likely prove so today if compared using the recent Cheminova product. I present these views as a way of dismissing any notions that Moeller and Rider has any fundamental flaw, if it can be accepted that malathion used in that study was at least as pure as 1962 vintage technical malathion, though purity of malathion



used in the study was not provided. If it were a more highly purified product, then to the extent that such culprit cholinesterase inhibiting impurities as malaoxon and isomalathion were reduced, the concern about relative human sensitivity would be to that extent more enhanced.

In view of these considerations, greater scrutiny of the rat cholinesterase data than was had at the November 6 meeting would be essential before a shift could be made from human to rat data as the basis for deriving an RfD. Along these lines I have the following to say. The Cheminova malathion technical product is said to be more pure than the former American Cyanamid product. Before the Committee accepts such claim, members should have in hand the Confidential Statement of Formulation for the respective products for direct comparison by the Committee. This is particularly important with respect to levels of cholinesterase inhibiting impurities. Cheminova has submitted data showing higher LD50 values for their product versus the American Cyanamide product, but LD50 may not be a good reflection of how products may compare at low levels of exposure based on cholinesterase data. LD50 values may be confounded by a host of adverse effects of the test material including cholinesterase inhibition brought on by trace impurities of cholinesterase inhibiting entities that do not require activation and thus become relatively more important at high doses of malathion where metabolic conversion of malathion to malaoxon becomes more saturated. Actually, I must confess to the committee that I very carefully compared the two product compositions awhile ago and there are reduced levels of malaoxon and isomalathion in the Cheminova product versus the American Cyanamid product, but I would question the relative effects of these these entities at low doses where metabolic conversion of malathion to malaoxon is less saturated.

In developing the protocol for the recently (1996) submitted malathion chronic/carcinogenicity study, the registrant was advised by our staff that 100 ppm, which the registrant was proposing as a low dose for the study, included principally in search of a NOEL for cholinesterase inhibition, would likely not be a NOEL for the blood borne cholinesterases. (Exhibit 2) It was explained that 100 ppm (lowest dose tested) was not a NOEL in the 1980 chronic/carcinogenicity study in the Sprague-Dawley rat, and likely would not be a NOEL in the new study. Nonetheless, the registrant elected 100 ppm as the low dose for the new study, partly predicated on their view that their product is more pure than the American Cyanamide product employed in the earlier studies. As it developed, after 3 months on test, statistically significant erythrocyte cholinesterase inhibition was observed in females, prompting a reduction of the low dose to 50 ppm for rats of both sexes for the duration of the two year study in search of a NOEL. (Exhibit 3) I should note at this point that this finding corroborated the finding in the Sprague-Dawley rat performed seventeen years ago using the American Cyanamid product. Subsequent to the three month time point, 50 ppm proved to be a NOEL for erythrocyte cholinesterase for both sexes. Firstly, what this says to me is that there is little if any improvement in the Cheminova product over that of the American Cyanamide product with respect to inhibition of erythrocyte cholinesterase at low doses, particularly those critical to setting the RfD for malathion. Secondly, in the DER for the new chronic/carcinogenicity study in the rat, additional cholinesterase information is called for in view of the absence of a NOEL for

cholinesterase inhibition among females at the 3 month time point. It is alleged in the DER that given the ability of organisms to adapt somewhat to cholinesterase inhibitors (see, for example, the recovery of erythrocyte cholinesterase inhibition for females at 500 ppm at 6 months in that study, Exhibit 4), there is no assurance that the enzyme would not have been inhibited at 50 ppm during the first three months, i.e. during a very critical time frame for exposure to a pesticide.

This is also very important in view of the facts that, a) malathion has a very shallow dose response curve (in my judgement there is very little difference between 50 and 100 ppm for an agent that demonstrates such a shallow dose response curve ranging up to 6000-12000 ppm), b) the human study demonstrated greater sensitivity for uncertain reasons and c) the number of animals assayed for cholinesterase activity, 10/sex, does not accord sufficient statistical power to clearly identify a NOEL at low but meaningful levels of inhibition. I must maintain at this point that a definitive NOEL for cholinesterase inhibition be determined over at least a three month period using large numbers of rats at doses that embrace those employed in Moeller and Rider (.11-.34 mg/kg/day) overlapping those of the lower dose range of the rat chronic/carcinogenicity study, say up to 20 mg/kg/day. To the extent that this end point will be employed in establishing the RfD for malathion, I view it imperative that this data be gathered.

In summary I consider it inappropriate to change the basis of the RfD for malathion from the Moeller and Rider human study to the recently submitted chronic toxicity/carcinogenicity study in the F344 rat, particularly without a definitive NOEL for cholinesterase inhibition over the first three months of testing in this case of the rat. Also, I recommend additional study to obtain a more definitive NOEL for cholinesterase inhibition at low doses in the rat

cc Jess Rowland

Brian Dementi  
Toxicologist, HED.

**COMMENTS ON THE POTENTIAL ROLE OF ALIESTERASES  
IN MALATHION TOXICOLOGICAL ASSESSMENTS**

As reported in several sources, e.g. Dauterman (1971) there are various carboxylesterases in the plasma and tissues of animals. Certain of these enzymes may play a significant role in the differential expressions of malathion toxicity. Dauterman cites references attesting to the presence of such enzymes as widely distributed in mammals and as having been found in the liver, kidney, serum, lung, spleen and ileum of the rat, mouse, guinea pig and dog. "This hydrolyase is present in certain malathion-resistant insects and it is reasonable to assume that resistance to malathion is at least partly due to carboxylase activity" (p. 139) Dauterman notes that the enzyme will hydrolyze only one of the two carbethoxyl groups on malathion.

Augustinsson (1959) presented research results and a good discussion on various types of carboxylesterases. Each type is actually a class or group of enzymes. The carboxylesterases might be defined as (1) aryl esterases - those which catalyze hydrolysis of aryl (aromatic) esters; (2) aliesterases - those which catalyze hydrolysis of both aliphatic and aromatic esters and; (3) cholinesterases - those which catalyze hydrolysis of cholinesters. There can be overlap in enzyme specificity.

The importance of aliesterases with respect to the toxicological profile for malathion might be explained as follows: unlike most organophosphates employed as pesticides, malathion has two carboxylester groups which in principle are vulnerable to hydrolysis catalyzed by aliesterases. Once one of these ester groups is hydrolyzed to yield a carboxylic acid substituent on the residual malathion molecule, the molecule loses its cholinesterase inhibiting capability and hence, loses its cholinergic toxicity. The structure of malathion is as follows:

\*\*\*\*\* STRUCTURES NOT PRESENTED HERE.....SEE FILE  
COPY\*\*\*\*\*

Reactions possibly catalyzed by aliesterases would yield, in principle, any of the following three molecules:

\*\*\*\*\*MOLECULES NOT PRESENTED HERE.....SEE FILE COPY\*\*\*\*\*

As reported, only one carboxylester group is cleaved on the molecule, which yields compound B resulting from action at the alpha carboxylester group. -Apparently this is the preferred site for the enzyme and once cleavage occurs, the carboxyl group generated precludes further binding of the molecule to aliesterase (Dauterman, 1971, p. 142). Of course, the product molecule will not inhibit cholinesterase. This is further substantiated in the work of Wilkinson (1976): "It is probable that the selectivity of malathion is directly related to the presence or absence of carboxylesterases in various species. Thus, carboxylesterase activity is found to be low or absent in several insect species susceptible to malathion (Kojima, 1961) and is usually high in malathion resistant strains or species (mot'oyama and Dauterman, 1974)." (p. 157)

Augustinsson (1959) examined the plasma of several species for all three types of esterases. Essentially, he found that while aliesterases are present in the plasma of many species, for example, rat, rabbit, horse, cat, guinea-pig, etc., this enzyme is absent from the plasma of the human, monkey and dog. Thus, to the extent that the enzyme is missing from human plasma, humans would lack this essential line of defense against cholinesterase inhibition by malathion, once oxidized to malaaxon. By contrast, the rat, rabbit, guinea-pig, etc. possess 'this capability in the plasma to detoxify malathion (cholinergically)'. This conclusion or rationale is further supported by the work of Main and Braid (1962) who demonstrated the essential absence of aliesterase activity in human serum, though finding it abundantly present in rat serum. These investigators were able to show that when serum aliesterase is inhibited in the rat using tri-o-tolylphosphate (TOTP), the acute toxicity of malathion was remarkably enhanced. For example, the LD50 of secondary standard malathion as reported by these investigations for the rat is, 1600 mg/kg. However, when administered one-hour post TOTP administration at doses inhibiting aliesterase activity, the malathion LD50 dropped to 35 mg/kg. Similarly, the LD50 of technical malathion dropped from 415 mg/kg to 7.5 mg/kg when TOTP was employed. The implication of this work is that with respect to anticholinesterase activity (and, hence, cholinergic effects) malathion toxicity may be greater in the human than in the rat. Indeed, according to Main and Braid, "The hydrolysis of malathion by aliesterase explains the vast difference between the toxicity of compounds, such as parathion and malathion.11 (p, 262). It should be noted that humans are not devoid of aliesterase activity. Human liver contains aliesterase activity, as does rat liver (Main and Braid, P. 257). Hence, according to Main and Braid: "It is difficult at this time to predict precisely the toxicity of malathion toward human beings on the basis of the detoxicating effect of aliesterase in the rat. The complete absence of aliesterase activity in human serum means that at least one important barrier to malathion poisoning present in the rat is absent in humans.11(p. 262) (A related reference is that of Ecobichon and Comeau, 1973). It should also be emphasized that the dog probably contains no serum aliesterase, but, as is true in the case of man, dog liver contains aliesterase activity. In fact, Augustinsson (1959) notes that esterase electrophoretic patterns of the plasma of the dog resembled that of human plasma (p. 584). "Human, monkey, dog, swine

and ruminant plasmata do not contain aliesterasell (p. 591) However, Augustinsson (pp. 584-85) appears equivocal as to whether there may be some aliesterase activity in dog plasma. The absence of aliesterase in dog serum is further substantiated by the work of Murphy and DuBois (1957).

"The serum of mice and rats was capable of detoxifying malaoxon, but dog serum exhibited no activity" (p. 815). These authors also report a 4-fold difference in this activity of the liver of male rats with respect to that of female rats, male's being more active.

The latter comments offered here with respect to the dog are ,designed in part to help assess the suggestion of Dr. A. A. Sadun (letter to B. Dementi, May 25, 1990) in which he advocates the dog as a better surrogate than the rat for man in the event ocular testing is pursued. The absence of aliesterase activity from the dog serum and the similarity of dog to man with respect to aliesterase profiles would support use of the dog over the rat in such testing. This would be expected to be true more so if anticholinesterase activity is important in the etiology of any -effects. However, the ocular organophosphate phenomenon might not be entirely cholinergic in nature, and malathion remains an organophosphate even after a carboxyethyl group is hydrolyzed. Nevertheless,based on this line of reasoning, it does appear the dog would be preferred to the rat as the surrogate. The articles cited above are appended. There is much noteworthy information in these articles. A thorough up-to-date search of the literature followed by review would be desirable in order to more definitively characterize the role of aliesterases in malathion toxicity. For now, information on aliesterases developed from these articles must be viewed as helpful.

#### ADDITIONAL NOTES:

(1) The, absence of aliesterases in human plasma could serve to explain the lower cholinesterase NOEL in humans (Moeller and Rider, 1962) than in rats. Hence, the malathion RfD based upon Moeller and Rider assumes more significance in view of the aliesterase distinction between man and the rat, for instance.

(2) The prolonged duration of malathion in human serum allows more time for conversion to malaaxon.

(3) Aldridge (1953) reported investigations showing that E600-esterase (an enzyme which hydrolyzes E600, (diethyl-pnitrophenyl phosphate) at the phosphate center to yield p-nitrophenol) is the same as arylesterse, the enzyme known to hydrolyze such arylesters as p-nitrophenylacetate, p-nitrophenyl propionate and p-nitrophenylbutyrate. The article appears to say that aliesterases' will also hydrolyze the latter carboxylic-acid esters, but is mute as to whether aliesterases with hydrolyze E600. To the extent that E600 esterase (arylesterase) will hydrolyze E600, it is behaving as a phosphatase; however, the article makes clear that arylesterases are different enzymes from the phosphatases. The article does not address the issue of how broad the phosphates - e activity of the arylesterases may be. Apparently this class will not catalyze

hydrolysis-of diethylphenylphosphate. Also, apparently aliesterases will hydrolyze p-nitrophenylactate, p-nitrophenylpropionate and p-nitrophenylbutyrate. It is doubtful that aliesterases will hydrolyze E600.

In any case, the author indicates the distinction between aliesterases and both aliesterases and phosphatases. This whole subject of hydrolysases in plasma and tissues of various species is a complex one, but of great interest. The literature discussed above represents but a partial effort. A thorough review of the literature followed by a comprehensive written review would be desirable.

Brian Dementi, Ph.D., D.A.B.T.,  
Toxicology Branch I  
Health Effects Division

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**JELLINEK, SCHWARTZ,  
CONNOLLY & FRESHMAN, INC.**

February 13, 1992

Ms. Joanne Edwards  
Product Manager (74)  
Special Review and Reregistration Division (H7508CO)  
Reregistration Branch  
Office of Pesticide Programs  
U.S. Environmental Protection Agency  
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Dear Joanne:

This letter contains our minutes on what transpired at our meeting on malathion with a member of your staff and two HED toxicologists on December 10, 1991. We would appreciate your concurrence with these minutes and the conclusions we reached concerning testing of malathion and malaoxon in chronic toxicity/oncogenicity studies. At the end of this letter, you will find a signature line for concurrence by HED toxicologists. If they do not concur with statements made in this letter that would affect the conduct of these studies, please inform us at your earliest convenience because testing is to be initiated early in 1992.

Those in attendance at the meeting included Mr. Jon Weis (Cheminova Agro A/S (Cheminova,)] and Dr. Judith Hauswirth (JSCF) representing Cheminova and Dr. Brian Dementi, Mr. Larry Schnaubelt, and Dr. Hank Spencer representing EPA/OPP.

Mr. Weis opened the meeting with a brief discussion of the transfer of the malathion registration in the United States to Cheminova solely. The only malathion technical product to be sold in the United States will be made by Cheminova. American Cyanamid will no longer be producing malathion for sale in the United States. As of December 31, 1991, the malathion task force no longer exists.

The discussion then turned to issues related to the conduct of rat chronic toxicity/oncogenicity studies on malathion and malaoxon and a mouse oncogenicity study on malathion. It was agreed that the malathion mouse oncogenicity study would be conducted for 24 months instead of 18 months, which would have been in accordance with the conduct of the NCI mouse oncogenicity study on malathion as requested by the Agency. Drs. Dementi and Spencer agreed to this because of the proposed performing laboratory's (International Development Corporation) historical control data base on studies of this length; Research and Development, however, they requested that they be informed if survival past 18 months becomes a problem in the

malathion study.

The two high dose levels (8,000 and 16,000 ppm) for this study have been previously agreed upon with the Agency. Dr. Dementi questioned why the low dose level was raised from 35 ppm, as originally proposed by American Cyanamid, to 100 ppm. Dr. Hauswirth stated that this was an American Cyanamid decision, that we would check into the reasoning, and that we would inform EPA of the reason as soon as we could.

The dose levels (20,000, 10,000, 5,000, and 100 ppm) selected for the chronic toxicity/oncogenicity study on malathion in the rat were discussed. Dr. Hauswirth presented the rationale for selection of the two high dose levels. The highest dose selected, 20,000 ppm, is the limit dose for studies of this type. The next highest dose level, 10,000 ppm, represents one-half of this dose level and would serve as the high dose for the oncogenicity study if mortality was excessive at the limit dose. The dose level of 5,000 ppm would serve as one-half of the top dose if mortality were excessive at 20,000 ppm. Dr. Dementi asked why 100 ppm was selected as the lowest dose level tested because in his opinion in past studies conducted on malathion 100 ppm was an effect level. Dr. Dementi stated he would prefer the lowest dose level to be 50 ppm. We agreed that we would look into this issue but informed EPA that the Cheminova technical malathion is less acutely toxic than the American Cyanamid technical. EPA was not aware of the differences in acute toxicity. Mr. Weis said that the comparative acute studies would be submitted officially to EPA and he showed EPA copies of the studies for discussion. It was suggested by Cheminova representatives that 50, 100, 10,000, and 20,000 ppm might be more appropriate dose levels for this study in light of Dr. Dementi's concerns about a NOEL for cholinesterase inhibition and the differences in toxicity between Cheminova's and American Cyanamid's technical products. EPA thought this might be more appropriate.

In addition, with regard to the rat chronic toxicity/oncogenicity study with malathion, Dr. Hauswirth noted that EPA had suggested that a 90-day range finding study be conducted prior to the chronic testing. Dr. Hauswirth suggested that a 28-day study be conducted, initially because of the already available toxicity information for malathion in the SpragueDawley rat and because this would cut down on the time it would take to initiate the two-year study. Dr. Dementi stated that he wants the chronic studies started on malathion as soon as possible and that he is very concerned about how long it has taken to initiate the studies. EPA and Cheminova agreed that this was a good approach to take and would be sufficient provided adequate data were provided for dose selection from the 28-day study results. If not, it was agreed that a 90-day study would be initiated perhaps in conjunction with neurotoxicity testing (a requirement of the draft data-call-in on malathion).

Dr. Dementi requested that we submit the methodology that will be used for cholinesterase activity determinations. Cheminova committed to providing this information and asked for a quick turnaround time on review. Dr. Dementi assured us that we would get a quick response and that the methodology should be submitted to Joanne Edwards. Drs. Dementi and



Spencer also asked that we inform them when the studies have been initiated and that we provide annual progress reports.

EPA and Cheminova agreed that the dose levels for the malaoxon chronic toxicity/ oncogenicity study have been previously approved by EPA. Dr. Dementi noted that ultimately dose level selection was the responsibility of the registrant. Mr. Schnaubeit informed Cheminova that the ocular toxicity and neurotoxicity testing guidelines are to be discussed at an EPA-sponsored workshop sometime in January and are therefore subject to change.

The meeting ended with a commitment from Cheminova to initiate the studies on malathion and malaoxon as soon as possible.

Diane Allemang  
JSCF & Co., Inc.  
Authorized Representative of  
Cheminova Agro A/S

cc: Brian Dementi  
Hank Spencer

Concurrence:

Dr. Brian Dementi

Dr. Hank Spencer

**STUDY TITLE**

Overview Of The Subchronic and Chronic Toxicity of Malathion

442797-01 I

**DATA REQUIREMENT**

U.S. EPA Pesticide Assessment Guidelines, Subdivision F:  
Toxicity Testing

**AUTHOR**

Jellinek, Schwartz & Connolly, Inc.  
1525 Wilson Boulevard, Suite 600  
Arlington, VA 22209-2411

**STUDY COMPLETED ON**

May 30, 1997

**SPONSOR**

Cheminova Agro A/S  
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Denmark

This page has been claimed confidential. It is releasable to persons who submit a signed "Affirmation of Non-Multinational Status" form.

**EXHIBIT 4**  
**Huntingdon**  
**Life Sciences**

Data Requirement

Test Guideline 83-5

STUDY NO. 90-3641

A 24-MONTH ORAL TOXICITY/QNCOGENICITY STUDY  
OF MALATHION IN THE RAT VIA DIETARY ADMINISTRATION

Final Report

VOLUME I OF XIV

Author: Ira W. Daly, Ph.D., D.A.B.T.

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Date: 27 February 1996

Page I of 5666

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November 20, 1997

Re: Ad Hoc Committee Meeting of November 13, 1997 on Malathion Issues

As a matter of the record, regarding the referenced meeting, this is to advise you that in spite of the good effort on your part to see that a fair and reasonable meeting was held, and I thought you did well, I do not consider the outcome satisfactory. The decisions made were very inadequate and not in the interest of the public health, as they compromise full pursuit of the understanding of the toxicology profile on this important and extensively used pesticide. No stone should be left unturned, given the enormity of human exposure to this cholinesterase inhibiting organophosphate. I shall comment on the topics that were the subject of the meeting in the order in which they were taken.

#### Retinal Anomaly in Acute Neurotoxicity Study on Malathion (MRID 43146701)

I have presented fully my views on this subject in written documents, which were available to the Committee, and will not restate these views at this writing. The fact remains that the Acute Neurotoxicity Guidelines (81-8) call for sequential histopathologic evaluations of specific tissues in lower dose groups when histopathologic findings are noted in the high dose group animals. It would appear to me that this requirement should be met in this Guideline even if but one lesion is observed in a particular tissue of the high dose group given the small number of animals (5/sex) in a dose group. This was not done in the study in question after the one bilateral retinal rosette was noted in a high dose male group. Now it is not a source of happiness to me to be perceived as one who over-assesses a study, and this is why I feel very awkward in defending this position. If the one incident standing alone had been identified among fifty or more animals in a group, surely I would not have pursued the matter, but in this case given the rarity of the lesion in historical data bases and the uncertainty as to the lesions microscopic anatomic features (retinal rosette is not an anatomic term and on the face of it, the term could be used to apply to any of a variety of underlying morphologic changes), I felt that as a matter of the record, our pathologist should provide anatomic characterization. Also, there was somewhat greater incumbency to require this assessment since it involved the retina, in view of the prevailing concerns over possible retinal effects of organophosphates in general and of malathion in particular. While I did not say so at the November 13 meeting, it is essentially self-evident that the assessment of the requested slides could be instrumental in determining whether to insist upon examinations of lower dose groups as mandated in the Guidelines. For example, this might be contingent upon whether the bilateral retinal rosette of the high dose male in the acute study is morphologically or anatomically the same as that of the unilateral rosette of a control rat in the subchronic neurotoxicity study.

Lastly, I believe the relatively minor decision to ask for a couple slides should be entirely within the purview of the reviewer, given what may be his peculiar perspectives on the subject, without having it go before a committee for approval. As I said, for the record, this issue remains unresolved if the slides in question are not submitted.

#### Relative Sensitivity of Females Versus Males to Cholinesterase Inhibition by Malathion

I presented to the Committee several comparisons of the level of cholinesterase inhibition for males and females from our Guideline and dose range-finding studies on malathion and malaoxon. Although the magnitude of differences between the sexes is variable across studies, there is more than adequate evidence to establish a greater sensitivity for

females. The ad hoc Committee did agree that sex-related differences are manifest, but did not concur with the proposition that differences may merit a correction factor to be applied to male (human) data used as the basis for the RfD. It should be noted at this point that the RfD for malathion, 0.02 mg/kg/day, which ostensibly protects the entire human population - men, women, boys and girls of all ages- employs a mere ten-fold safety factor as applied to experimental data obtained on humans (men only). In the absence of such data for women and youths, in my judgement a larger safety factor than ten should be employed, particularly in the face of evidence that females are more sensitive to malathion than males as assessed in laboratory animal studies, and where studies of organophosphates in general suggest young individuals to be more sensitive. According to the 1988 malathion registration standard: "The Theoretical Maximum Residue Contribution (TMRC) for the U.S. population average is 0.1014 mg/kg/day, occupying 505% of the PADI. For children 1 to 6 years of age, the TMRC occupies 1133% of the PADI. The TMRC is based upon current tolerance levels and an assumption that 100% of the sites are treated. Actual dietary exposure may be much lower." (p.32) The point is that a much higher percentage of the PADI is consumed, or was so in 1988, than is to be desired, which places an enhanced scrutiny upon the reliability of the RfD in protecting real people.

Unfortunately I did not have the time before the meeting to provide study by study estimates of such correction factors, but am certain that a legitimate correction factor, whatever it is, would be of such magnitude that it should not be ignored, especially in view of the small safety factor used for the existing RfD. Additional study in animals may be necessary to properly identify the correction factor. Realizing that a sex-related differential sensitivity exists, unacceptable in my opinion is the Committee's out of hand rejection of the argument that a meaningful ratio exists without first obtaining some numerical estimates of that ratio of sensitivity from the data currently in hand. Indeed, I had anticipated that an outcome of the meeting would be a Committee recommendation that such estimates be computed for subsequent consideration.

#### Testing for Effects on Learning/Memory

Again, available to the Committee were various documents presenting arguments pro and con that findings with malathion on learning/memory at very low doses in a published work, Desi et al. (1976), are of sufficient validity and concern to require Guideline testing of malathion for these effects. In addition to explaining to the Committee that the published work shows that malathion at doses of 38-75 mg/kg/day in a subchronic study elicited effects on learning/memory, EEG and EMG, as contrasted with no neurotoxic (motor activity, FOB parameters) effects in the Guideline subchronic neurotoxicity study at doses up to 1575 mg/kg/day, I had recommended that a Guideline test of learning/memory be required for malathion. The Committee rejected this recommendation on the grounds that Desi et al (1976) is not a reliable study. This criticism of the study was maintained in spite of many findings in the study that affirm its veracity. Of these I mentioned the facts that the stated purpose of the authors was to assess the effects of malathion at subclinical levels on sensitive neurotoxicity parameters including learning/memory; 95% malathion (American Cyanamid) was used; the authors affirmed the absence of clinical signs which was consistent with the low but meaningful level of cholinesterase inhibition; cholinesterase activity was remarkably well evaluated in the study, including assessments of plasma, erythrocytes and brain regions, where the findings were consistent with those of the Guideline subchronic neurotoxicity study (which in turn enhances the credibility of the published work), and adverse effects of malathion on kidney tissue in *in vitro* kidney tissue cultures being somewhat consistent with or supported by chronic nephropathy as the cause of increased mortality (100% and 74% in the high and penultimate doses, respectively) in the 1996 chronic toxicity/carcinogenicity study in the F344 rat. Furthermore, the authors of the study affirm in the text a real effect of malathion on learning and memory as assessed in their study.

The Committee members were mute with respect to acknowledging any of these facts as supporting evidence of the



work by Desi et al, but persisted in criticizing the study on the grounds that the effects on learning/memory in terms of errors made by rats in maze studies were small, not dose related between 38 and 75 mg/kg/day; that statistics were ill defined and that it would be surprising for malathion to exert such an effect at such low dose levels. I endeavored to explain that findings were in fact not small in terms of differences in errors made in dosed groups versus controls. I also offered my opinion that 38 and 75 mg/kg/day, when compared on the shallow dose response for malathion are actually not very different, and that brain cholinesterase inhibition was 20% in the two groups at 21 days, the time at which learning/memory was affected. These two observations would point to similar responses on tests of learning/memory, and thus the absence of a dose response as noted. I also explained from an earlier work by Desi et al, which the authors cited as background for methodology, that bar graphs in that study were said to be standard deviations, which if true in the 1976 study would mean that differences between controls and dosed groups on errors made in the learning /memory test would be statistically significant. In spite of these findings, plus the EEG and EMG data affirming a neurological effect of the test material at these dose levels, and in view of the fact that the Guideline subchronic neurotoxicity study was not designed to assess learning/memory, EEG or EMG effects that could refute the findings in Desi et al, the Committee categorically rejected the Desi study as of any relevance. In fact, I recall saying to the group, "It's as if Desi does not exist?", whereupon I was responded to in the affirmative. In my judgement, this qualifies as an authoritarian rejection of data the Committee failed to refute. I maintain that Desi et al (1976) in spite of its deficiencies is of sufficient quality that its conclusions, particularly with respect to the effects of malathion on learning/memory, mandate verification through proper Guideline testing procedures, which are available. As to the question of the "small" effect on errors made by rats in the learning and memory aspect of Desi, et al, one might ask, what is small? Imagine a high school student taking his algebra exam, on which his grade would be say 97, other things being equal, but under the influence of a xenobiotic he was exposed to, his score turned out to be 92 due to a few additional errors he made. Now a 92 (B) is a very good grade, but not quite as good as the grade he deserved 97 (A). One might say this is a small difference, but who would argue that is to be ignored?

I have concerns about the legitimacy of the opportunity presented to me to go before an unbiased ad hoc committee. I had reservations before the November 13 meeting that I should even pursue the matter. This concern was born out by the following episode that occurred at the meeting. As you will recall during the meeting, at the precise moment that we completed our deliberations on the second topic, one Committee member, arriving late, voted on the issue. In fact, as I recall, you commented at the time that so and so is voting even though she was not present during the discussion. From my perspective, her vote was more than improper in that it conveyed the impression, whether rightly or wrongly interpreted, that the Committee's conclusions were foreordained, and that my opportunity to be heard at this meeting was a mere formality. When I came to item three, my presentation was compromised in the psychological or motivational sense, given what had previously taken place. I could see "The handwriting on the wall" and thus the futility in proceeding further on what was really the most important of the three issues.

In my view, minds had been made up, and I felt nothing I said would matter before this Committee. Indeed, I came preciously close to calling off any further discussion, but felt that would be of no avail either, as people might then say "well, you had your chance", as if this were some kind of real and legitimate peer review. I am convinced it was so in name only. The bottom line to all this is that another forum for peer review of these issues is required, bearing in mind the importance of this subject to the public health. People composing a true peer review committee should be experts in the field, but at the same time should not have personal vested interest in HED.

Brian Dementi  
Toxicologist, HED

cc Jess Rowland

November 25, 1997

RE: Malathion RfD

It is my intent here to comment further on certain issues before the Hazard ID SARC of November 6 and the Ad Hoc Committee meeting of November 13, 1997, with particular reference to the RfD for malathion.

In my memorandum to you of November 10, I endeavored to explain why the cholinesterase data in the recent chronic toxicity/carcinogenicity study of malathion is inadequate to define a NOEL for female F344 rats. As a remedy, I recommended a definitive three month assessment of cholinesterase inhibition in the rat. In my judgement, until such data are available, a gap exists with respect to the identification of a NOEL for the first three months of exposure to malathion, and, hence, proper data do not exist in this study upon which to poise an RfD. This being true, and to the extent that the Moeller and Rider (1962) study, performed in humans, may continue to be used as the basis for the RfD until proper rat data are obtained, the following comments are relevant.

At the Ad Hoc Committee meeting, when discussing the topic of greater sensitivity of females to cholinesterase inhibition by malathion, I expressed the view that for studies wherein cholinesterase inhibition was obtained in but one sex, as is true in Moeller and Rider where only male volunteers were tested, that a greater than the normal uncertainty factor (UF) of 10 should be applied. As I recall, this was not affirmed by any one at the meeting. I suspect no one felt sufficiently certain to render a definite opinion. In any case, I believe this is a question requiring an answer. I do not have the time to search the records, but I believe the answer should be readily available in the minutes of past RfD meetings, and should be a well recognized operating principle for the RfD Committee. I have just by chance reviewed the 1997 Registration Eligibility Document (RED) toxicology chapter for carbofuran, and I find in the case of the RfD that the Agency applied a UF of 100 to the NOEL for cholinesterase inhibition in male volunteers. Quoting from that RED chapter: "An uncertainty factor (UF) of 10 was applied to account for intra-species variability. An additional UF of 10 was applied to account for study deficiencies (use of limited number of subjects, few subjects/dose and **use of males only** (emphasis added)". Please be aware that Moeller and Rider, in addition to being a study in males only, has its inadequacies also (e.g., limited number of subjects, purity of the test material not provided, interpretation of low and mid dose effects somewhat confounded by co-administration of EPN).

In my memorandum to you of November 20, I quoted from the malathion registration standard, passages revealing how high the TMRC is (or was in 1988) when based on the RfD of 0.02 mg/kg/day, derived from Moeller and Rider with a UF of only 10. The Committee should be aware that at an earlier time point, a UF of 100 had been applied to Moeller and Rider, at which time the RfD was thus 0.002 mg/kg/day. Also at that time the TMRC was about 5000% of the PADI. At some point in time, and I don't have the details, I would estimate around 1987-90, the UF was reduced from 100 to 10, for reasons unknown to me.

I recommend that your Committee seek the historical record on the setting of the RfD for malathion, and make your own independent assessment of its reasonableness, as this is the moment in time for reconciling the RfD with the facts at hand. On the face of it, if a UF of 100 is appropriate for carbofuran for the reasons given, an explanation should be forth coming for the use of only 10 in the case of malathion. Please understand I am not saying a satisfactory explanation does not exist, but let us see it. I must maintain the view that when a UF of only 10 is employed, it is imperative that the study in question incorporate data on both sexes.

In summary, in my view proper data on cholinesterase inhibition in rats are not available at this moment to justify replacing the Moeller and Rider human study as the basis for the RfD for malathion. Furthermore, in the absence of cholinesterase data on women, the UF as applied to the Moeller and Rider human (men only) data should be revised upward from the 10 which is currently employed.

Brian Dementi, Ph.D.  
Toxicologist/HED

cc Jess Rowland  
George Ghali

Comments on December 4, 1997 draft report of malathion Hazard ID Committee meeting of November 6, 1997. The following is the best I am able to produce given the constraints of time and the complexity of the subject.

Comments on the various endpoints are presented as follows in the order in which they appear in the draft report.

I Introduction (p. 1) O.K.

## 11 Hazard Identification

A. Acute Oral (one-day): For this endpoint, the Committee concluded that the 50 mg/kg/day dose is appropriate for acute dietary risk assessment. This endpoint is based upon decreased maternal body weight gain in the malathion developmental toxicity study in the rabbit (MRID 152569). In support of this, the draft Hazard ID Committee Report (HIDR) cites the DER for the rabbit developmental toxicity study as showing a LOEL/NOEL of 50/25 mg/kg/day. However, it must be recognized that the DER concluded this conditionally upon receipt of Appendix III (DER p. 7), which contains individual animal data and was not included with the study MRID. This Appendix was submitted later as part of MRID 40812001, which includes the full study as well. I am not certain whether this individual data was evaluated by anyone in HED. It was explained in the Der (p. 6) that the non-statistically significant maternal body weight gain decrease at the low dose (25 mg/kg/day) could not be adequately evaluated due to the absence of individual animal data located in the missing Appendix III. As cited in the HIDR (p. 3), mean body weight gain during days 6-18 of gestation were 0.19, 0.06, - 0.03 and - 0.03 kg at 0, 25, 50 and 100 mg/kg/day, respectively. In order to evaluate statistically the numerical decrease at the low dose level vs. Control, i.e. 0.06 vs 0.19 kg, the individual data would be needed. Furthermore, the DER claims that the decrease seen at the low dose was principally accounted for during days 6-12 and that during days 12-18 the low dose dams actually gained more weight than controls. According to the study report, body weight gain during gestation days 6-12 were 0.08, -0.04, - 0.02 and -0.06 kg for control, 25, 50 and 100 mg/kg groups, respectively, where none of the dosed groups were reported as statistically significant with respect to control. (MRID table 3, p. 18).

In my opinion the data should be more closely examined before concluding where the LOEL/NOEL lies in this study, particularly if this end point is to serve as the basis for acute dietary risk assessment.

The HIDR says that there were no decreases in body weight gain at 50 mg/kg/day in the Range-Finding study. (P. 5). However, inspection of doe body weight gain data in the range-finding study shows body weight was not significantly altered at any dose level up to and including the highest dose of 400 mg/kg. (MRID 152569, table 3, p.16). Evidently, the reasons for this lack of a finding of an effect on body weight gain include the small number of animals employed and the high variability in body weight data. I do not see how this data can be cited in support of any conclusion with respect to effects of the test material on doe body weight. Furthermore, before concluding that a single dose as high as 50 mg/kg would not elicit a meaningful biological effect one should have cholinesterase data over several days following that single dose. In a journal publication mentioned in DER #11, p. 11 provided the Committee, it is noteworthy that as assessed in the Sprague-Dawley rat where malathion (American Cyanamid 95% t.a.i) were administered intraperitoneally at single doses of 0, 25, 50, 100 or 150 mg/kg, avoidance behavior was significantly impaired 1 hour after injection with 50 mg/kg and above. There were no clinical signs observed over a 24-hour post-dosing period at any dose excepting one rat in ten at the 150 mg/kg group, which exhibited tremors.

Cholinesterase inhibition was significantly inhibited only at 100 and 150 mg/kg during the 24-hour period, so the author concluded that low doses of malathion may disrupt behavior without significantly reducing cholinesterase activity [Kurtz, P. J. (1977) Dissociated Behavioral and Cholinesterase Decrements following Malathion Exposure, *Toxicol. Appl. Pharmacol.* 42, 589-594]. The behavioral effect found in this study was remarkable as observed at the 1 hour post-dosing time point, but was not observed at 4 or 24 hour time points.

I do not accept that a developmental toxicity study provides sufficiently rigorous toxicologic data to serve as the basis for defining this critical end point. The absence of cholinesterase assessments in particular in these studies should preclude their use as the primary source of information for an end point as important as that for use in acute dietary risk assessment.

#### Acute Dietary Risk Assessment

The HIDR claims that the 10X factor to account for increased sensitivity of infants and children required under FQPA should be removed. This is rationalized on the grounds there is no evidence in the reproduction and developmental toxicity studies of increased sensitivity of developing and young animals. In the rabbit developmental toxicity study doses administered during gestational days 6-18 were 0, 25, 50 and 100 mg/kg/day. Similarly in the rat developmental toxicity study (MRID 41160901) doses administered during gestational days 6-15 were 0, 200, 400 and 800 mg/kg/day. We concur that in neither of these studies was there any evidence of increased sensitivity of the developing organisms with respect to the dams, insofar as the parameters evaluated were concerned. There is a serious question, however, whether such parameters are adequate to detect critical end points. The lowest dose used in both of these studies are well above those that inhibit cholinesterase in adult rats and rabbits. In the absence of cholinesterase assessments or clinical signs in the developing organisms versus those of the maternal animals, it is simply not possible to affirm that the developing organisms were not more adversely affected than the maternal animal. I am of the opinion that cholinesterase inhibition could have been more remarkably inhibited in selected developing tissue of fetuses, and furthermore, a given level of inhibition may be more deleterious in various ways in developing organisms that would not be found in the limited set of end points evaluated in developmental toxicity studies. On the face of it, though the developmental toxicity study is useful in detecting possible developmental anomalies, its capability is not sufficient to address possible cholinergic effects or cholinesterase inhibition, as these very fundamentally important parameters are simply not evaluated.

In the case of the reproduction study (MRID 41583401) concentrations administered via the diet for two generations were 0, 550, 1700, 5000 and 75000 ppm. The low dose concentration in this study translates to 43 mg/kg/day for males and 51 mg/kg/day for females. The HIDR states that pups were no more sensitive than adults on the basis of such parameters as body weight, mortality, clinical signs. It is my observation that doses of 43-51 mg/kg/day and above would have resulted in cholinesterase inhibition, given the facts that the enzyme has been shown in other subchronic studies or time intervals to be inhibited at much lower doses, in fact. It is not particularly surprising that clinical signs were not observed except at the highest dose. In terms of clinical signs, rats tolerate cholinesterase inhibition borne of malathion exposure remarkably well. As in the case of the developmental toxicity studies, the question is whether a differential inhibition between pups/young animals and adults would have been observed, and whether young individuals are more or less sensitive in terms of behavioral effects (a term that embraces many types of end points). These parameters are not evaluated in these types of studies. So I must reiterate the opinion that developmental and reproduction studies while perhaps adequate to assess the effects of chemicals on the parameters of primary interest in those studies, namely developmental and reproductive effects, such studies are not of the character needed to differentiate relative sensitivity of young and mature animals to satisfy FQPA concerns. The absence of cholinesterase assessments is a most fundamental road block for this use of these studies. The elimination

of the 10X factor cannot be justified except on crude and therefore risky terms from the public health perspective. There is evidence from various studies that young and developing animals have an enhanced sensitivity to cholinesterase inhibitors in general, attributable to cholinesterase inhibition [Pope, C. N. and Chakraborti, T. K. (1992) Dose-Related inhibition of brain and plasma cholinesterase in neonatal and adult rats following sublethal organophosphate exposures. *Toxicol.* 73, 35-43]. Therefore, there is incumbency to demonstrate that young animals are not more sensitive than adults to the effects of malathion on that very basis, namely, cholinesterase inhibition and behavioral consequences, which were not assayed in the very studies cited to rule out the possibility of greater sensitivity of young individuals.

It is a curiosity that in HIR pp. 13-14 under the topic of Determination of Sensitivity, mention is made of the fact that cholinesterase data were not obtained for maternal animals nor their offspring or fetuses in the reproduction and developmental toxicity studies, without any attendant discussion of the implications of this lack of data. I believe the implications are precisely those expressed above, which is that without such data it cannot be said that young animals are no less sensitive than adults to the effects of malathion, and, hence, the elimination of the FQPA required 10X factor would be without justification.

B. Chronic Dietary [Reference Dose (RfD)]: This portion of HIR shows the calculation of an RfD based upon plasma cholinesterase inhibition in the recent F344 rat chronic toxicity/carcinogenicity study (MRID 43942901). The problem I have with this is that it does not address the failure of that study to identify a NOEL for erythrocyte cholinesterase inhibition among females during the first three months of testing. My arguments are discussed in my November 10, 1997 memorandum to Clark Swentzel, Chairman of this Committee. I will not take the time to reiterate those views here, except to emphasize the importance of obtaining a definitive NOEL for cholinesterase inhibition as explained in the memo cited. Given the facts that erythrocyte cholinesterase was inhibited in female rats at 100 ppm and 500 ppm at the three month time point, but not at the 50 ppm or 500 ppm levels at the six month time point is inexplicable. Possible explanations are that there is adaptive recovery post three months (in which case 50 ppm is not a definitive NOEL for that initial three month period, a critical time frame) and too few animals were employed to obtain good cholinesterase data in view of the shallow dose response for malathion. Such possible explanations support conducting a definitive cholinesterase assessment over a three month time point using adequate numbers of rats to provide statistical resolution. Another possible explanation is flawed cholinesterase methodology, which if true may be a more fundamental problem not peculiar to malathion. The point is that until a NOEL for cholinesterase inhibition among females has been determined via a definitive study, the transfer of the RfD from the Moeller and Rider study in my opinion lacks adequate support.

The HIR (p. 6) claims that the NOEL of the 2-year study is supported by the 90-day study. If this is in reference to the subchronic neurotoxicity study (MRID 43269501), it is true a NOEL of 50 ppm was found over the 90-day period, but that study employed but -5 rats/sex/group at each time point and had no other dose group between 50ppm and 5000 ppm that would demonstrate the ability of the study to detect cholinesterase inhibition within that large range. Furthermore, plasma cholinesterase inhibition is so imprecise in that study that it is questionable whether 5000 ppm or even 50 ppm is a NOEL in either sex, which underscore the need for a study on a large number of animals to obtain a definitive NOEL for cholinesterase inhibition.

In the mouse carcinogenicity study (MRID 43407201) there is no NOEL for liver histopathology in male mice, where the LOEL is 100 ppm (17.4 mg/kg/day). This study awaits a Pathology Work Group evaluation.

Chronic Dietary Risk Assessment : HIDR (p. 6) says that the Committee determined that the 10X factor should be removed. The reasons cited are the same as those for dropping the 10X factor from the acute risk assessment, namely the reproduction and developmental toxicity studies do not show a greater sensitivity of offspring or fetuses. To this I respond with the same arguments presented above in the case of the acute risk assessment, which is that it is not justified.

#### C. Occupational/Residential Exposure

1. **Dermal Absorption:** O.K.

2. **Short-Term Dermal - (1-7 days)** : O.K.

3. **Intermediate-Term Dermal (7 Days to Several Months)** : O.K.

4. **Long-Term Dermal (Seven Months to Life-Time)** : O.K.

5. **Inhalation Exposure (Any-Time Period):** The executive summary provided for the subchronic inhalation study is correct. I should emphasize that hyperplasia of the olfactory epithelium was described as locally extensive and that the olfactory/respiratory epithelial junction was severely affected in most animals. This means at all doses and there was no NOEL. The HIDR claims that since this study is the only inhalation study available in the toxicology data base, the LOEL will be used for short - intermediate - and chronic inhalation risk assessment. I view this as quite a burden for a study without a NOEL for both cholinesterase inhibition and nasal hyperplasia, but I have the greater concern for the hyperplasia aspect. **It is my opinion that this Committee should mandate a new inhalation study designed to identify a NOEL for histopathology of nasal tissues.** I say this not only because there was no NOEL, but because the hyperplasia is described as severe. There is a rational basis for a remarkable effect of malathion in particular on the olfactory epithelium, which is discussed at length in the DER for the recent malathion F344 chronic toxicity/carcinogenicity study (MRID 43942901). Briefly, the sensitivity of the olfactory epithelium to malathion rests with the remarkable metabolic capability of this tissue, as well as the unique structure of malathion as a diester of a dicarboxylic acid which may be hydrolyzed in the olfactory epithelium to yield carboxylic acids. The metabolic capability of the olfactory epithelium has been hypothesized as critical to the maintenance of acuteness of olfaction via the elimination of foreign materials including odorants. Given these factors which may explain the remarkable effect of malathion on the olfactory epithelium, in concert with the severity of the effect, as well as not knowing the time of onset of hyperplasia, I consider the application of a mere UF of 3 to cover for the lack of a NOEL to be entirely inadequate. I say this in view of both the smallness of the UF chosen, and an operating philosophy which in lieu of weighing the significance of the finding, simply invokes a UF without offering any explanation as to why 3 is adequate, or why another study should not be required.

The April 27, 1995 HED memorandum conveying the DER to the Product Manager says among other things: "The question of carcinogenicity as it may relate to the microscopic lesions of the nose and larynx will be addressed in a separate memorandum." To my knowledge such a memorandum remains outstanding, and this very important issue has not been addressed.

#### D **Margin of Exposure for Occupational/Residential Exposures**

(1) MOE for Dermal Exposures: see comments as before on the use of reproduction and developmental toxicity studies to rule out the possibility of enhanced sensitivity of young animals.

(2) MOE for Inhalation Exposures: As stated above, I do not support the use of the UF of 3. Again I find unmerited the claim that: "No FQPA factors are required since there was no indication of increased sensitivity in the offspring of rats or rabbits to prenatal exposure to malathion.", lacking cholinesterase data or behavioral effects assessments.

## **E Recommendation for Aggregate Exposure Risk Assessments**

No additional comments

## **III. FQPA Considerations**

### **1. Neurotoxicity Data**

In the case of the acute neurotoxicity study, concerning bilateral retinal rosette observed in one male rat, the statement might be improved somewhat in its meaning by saying that the one rat in which it was observed was from among but five males examined histopathologically in the high dose group, and that none were examined in lower dose groups. Also, concerning the acute and subchronic neurotoxicity studies mentioned, I would cite my memorandum of November 20, 1997 to Clark Swentzel as detailing comments I might otherwise offer here.

### **2. Determination of Sensitivity**

No further comments on the developmental and reproduction studies.

## **VII Data Gap(s)**

Roman numerals go from III to VII in the HIDR.

From my perspective, the following are data gaps:

1. Carcinogenicity Study in B6C3F1 Mice (MRID 43407201) :  
Pathology Working Group assessment for liver tumors;  
Histopathology assessment of nasal tissues.
2. Combined Chronic Toxicity/Carcinogenicity F344 Rat Study (MRID 43942901) : Pathology  
evaluation/revaluations of various tissues.
3. Subchronic Inhalation Study in Sprague-Dawley Rat (MRID 43266601) : resolution of no NOEL for nasal tissue  
histopathology, which was severe at the lowest dose and present in essentially all rats of both sexes; recommend a  
new and longer term study to address the absence of a NOEL and potential carcinogenicity by the inhalational route.
4. Developmental Toxicity Study in the Rabbit (MRID 152569) : submission of Appendix III followed by statistical  
treatment of the individual data to affirm the NOEL for body weight effects in dams particularly over days 6-12 of  
gestation.
5. Acute Neurotoxicity Study in the F344 Rat (MRID 43146701): submission of selected retinal tissue slides as  
called for in the DER.



6. Subchronic Neurotoxicity Study in the F344 Rat (MRID 43269501) : submission of a guideline behavioral test yet to be specified.
7. Three-month cholinesterase assay in the rat to determine a definitive LOEL/NOEL for malathion.

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